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FURTHER OBSERVATIONS ON AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF THYROID GLAND IN THE GUINEA-PIG*

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In the previous papers from our laboratory Cora Hesselberg [1] and the writer [2] analyzed the factors active in the transplantation of the thyroid gland of the guinea-pig in cases of auto-, syngenesio-, homoio- and heterotransplantation. In further studies, we attempted to obtain additional data through the study of multiple and successive transplants.[3,4] While these previous investigations establish some basic facts as to the interaction of tissues and prove the existence of quantitatively graded toxic substances in the interaction of tissues under certain conditions, still many problems remain as yet unsolved and in a series of investigations we shall take up the analysis of these problems. In this first communication we shall briefly discuss the result of some further observations on auto- and homoiotransplantation of the thyroid gland in the guinea-pig.

A. AUTOTRANSPLANTATION

1. If we examine an autotransplant of thyroid tissue in the guinea-pig about five months after transplantation, we find that the transplant has assumed almost completely the character of the normal, not transplanted, thyroid gland. The thyroid is surrounded by the fat of the subcutaneous tissue. The acini on the whole are large and contain good colloid which is either adherent or retracted;

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it is dense and solid and takes the eosin stain intensely. The peripheral acini especially are large and lie close together. In certain places the cells separating adjoining acini may degenerate (pyknotic nuclei) and then dissolve. In this way neighboring acini may coalesce, the remaining spurs still indicating where the walls broke through. Some acini perish entirely and thus a certain amount of colloid may gain access to the interstitial tissue. It is possible that the hyaline material, which is found occasionally in the stroma of the thyroid in the guinea-pig, originates in this way; also similar material observed in certain cases in the human thyroid. We may have to deal in these instances with mixtures of colloid which have found access to the interstitial and connective tissue structures. In such transplants, dense connective tissue bundles separating the acini, as well as collections of lymphocytes, are lacking. In what corresponds to the original center of the transplant, we find only small strands of loose connective tissue.

2. If we compare with this end stage earlier conditions, such as are found for instance between the fifteenth and eighteenth day after transplantation, we notice considerable differences between the two stages. In the beginning, the parenchyma is not yet so fully developed as later; however, the principal difference concerns the center of the transplant. Here we find in the earlier periods after transplantation an area of dense hyaline fibrous tissue often surrounding some vessels in addition usually to the large capillaries and the edematous connective tissue which are situated especially at the inner edge of the thyroid ring. It is obvious that in the course of time this fibrous material must gradually disappear. As to the mechanism through which this occurs, it is at least probable that it is due to the activity of fibroblasts. We can see at these earlier periods a zone of fibroblasts around capillaries and often containing blood pigment cells; they surround and penetrate this hyaline fibrous material and substitute for it a cellular material which is gradually absorbed. It is possible, however, that in certain cases the central fibrous material is later pushed out into the surrounding tissue through a cleft in the ring of thyroid acini.

3. While, as stated previously, the center of the autotransplant consists usually of loose areolar connective tissue, capillaries, layers of fibroblasts and the mass of hyaline fibrous tissue which is being gradually absorbed, there is quite frequently an additional constit-

uent found in the center of the transplant, namely, fat tissue. It appears that this has been pushed into it from the surrounding fat, perhaps as a result of pressure. In one case we observed, as we recorded previously, that fat was included even in the center of an acinus. The greater part of this fat must also gradually disappear, because at later periods the autotransplant consists almost exclusively of thyroid tissue with only a very slight admixture of other tissues. As we stated in our preceding paper, fat tissue transplanted with the thyroid may cause a partial necrosis of the thyroid tissue at one point and here the ring of thyroid tissue is incomplete. This defect likewise disappears later.

In this connection we may mention that in the transplanted fat tissue, solution processes may take place, as the result of which cavities develop which may be multiple. Furthermore, in the case of autotransplantation there is occasionally found an epithelioid and giant cell reaction in the fat tissue, although this seems to be less frequent here than in the case of homoiotransplantation.

4. In addition to the fibrous tissue and fat which are gradually and more or less completely eliminated from the autotransplants of thyroid, there is present within the first two weeks the central necrotic material which is being organized by connective tissue; within this necrotic tissue there is often a variable amount of extravasated blood. Such extravasated blood may also find its way into the lumen of some acini where it becomes mixed with the colloid. Again this blood also gradually disappears. When it is situated in the acini, it is usually taken up by mononuclear phagocytes and we may assume that they contribute to its removal. There may occasionally be seen some epithelioid and giant cells around the blood coagulum in the center of the transplant as well as in the individual acini, but usually giant cells are absent in the organizing tissue. Phagocytes are also found in the colloid, situated within the acini, at least in the first five or six weeks following transplantation; and the colloid may, in part at least, be destroyed through the activity of such cells within the lumen of the acini.

5. There are present in the early period following transplantation isolated lymphocytes or some very small collections of lymphocytes scattered here and there either in the central connective tissue, in the capsule around the transplant or around the capillaries. Some isolated lymphocytes may also occasionally be seen between the

acini. These small lymphocytic collections are probably due to disturbances of a non-specific character connected with the transplantation and the healing of the tissue in a strange environment and cannot be mistaken for the very specific lymphocytic reactions which are due to the influence of homoio-toxins. Quantitatively the difference between the two kinds of reactions is very marked. Furthermore, the very slight and non-specific collections of lymphocytes in autotransplants apparently disappear completely or almost completely in later periods. At least they were lacking in the specimens which we examined five months after transplantation.

6. Localized infection, which occurs occasionally in the autotransplant, usually increases the amount of necrotic material found in the central areas of the transplant or it may cause necrosis elsewhere in the thyroid ring. We are especially likely to find as a result of infection an increase in the fibrous tissue at the inner or outer margin of the thyroid ring. As we have seen previously under normal conditions, the inner margin of the thyroid ring is usually covered by loose vascular connective tissue or by strands of fibroblasts. With the thyroid, the parathyroid generally remains well preserved in the case of autotransplantation and the parathyroid cells may often undergo mitotic division.

7. In autotransplantation, the transplant in the course of time more and more approaches the normal non-transplanted tissue. The abnormal reactions on the part of the fibroblasts and lymphocytes are called forth by certain more or less accidental conditions. These reactions tend to overcome and eliminate the conditions which called them into being. After this has been accomplished, all activity on the part of these cells ceases and they largely disappear. Blood vessels assume their normal size. Evidently the metabolism of thyroid tissue is adjusted in such a way that connective tissue and blood vessels are kept in a resting, inactive condition, just sufficient to permit the satisfactory functioning of the thyroid tissue. The thyroid tissue and its stroma are adjusted to a mutual state of equilibrium. Under ordinary conditions the tissues tend to return to this equilibrium. The case is different if other than autotransplantation is carried out. Then specific toxins interfere with the attainment of this equilibrium.

B. Homoiotransplantation

In former papers Dr. Hesselberg and the writer have defined the typical character of homoiotransplants of thyroid and parathyroid in the guinea-pig. We have also mentioned that it is possible to obtain the typical homoio-reaction on the part of lymphocytes, connective tissue cells and vessels in animals of various ages and in pregnant as well as in non-pregnant animals. However, several important problems were left unsolved: (1) Certain variations occur in the intensity of reaction after homoiotransplantation. It is necessary therefore to determine the range of variations which do occur; in particular it is desirable to determine how far the reactions in the case of homoiotransplantation may approach those of syngenesio- and even of autotransplantation. (2) It is important to determine how far such factors as age of donor and host, or various stages of pregnancy affect the intensity of reaction. The investigations on which we shall report here are concerned with these questions. The second part of the problem, however, cannot yet be regarded as definitely solved, although our results suggest certain conclusions in this direction.*

I. From 20 to 40 days after transplantation. We shall first discuss the range of variations which we found at a stage of transplantation when all the reactions were well developed, namely, at a period of from twenty to forty days after homoiotransplantation of the thyroid and parathyroid. In general, we can distinguish three degrees of reaction. Type I: In the severest reaction, no thyroid tissue is left twenty days or later following transplantation; only some fibrous tissue, perhaps with some lymphocytes, is found. Type II: In the second type of reaction, the thyroid is to a large extent destroyed, but some remnants are left. There is dense fibrous tissue in the center of the transplant with some acini which instead of containing a regular lumen with colloid, merely contain clefts, although there may be a few acini seen with colloid. In the lumen also of certain acini are found some desquamated epithelial cells which are mostly degenerated. There is much lymphocytic infiltration in and

* In addition to the complexity of the variable factors which are determined by the character of the problem there enters the possibility that, in certain cases through oversight on the part of the attendant of the animals, animals may have been mixed up. While we made efforts to prevent such occurrences, still we cannot be certain that in some cases they may not have taken place. Only if relatively considerable means are available for the care of animals, will it be possible to exclude such accidents.

around the acini, and the lymphocytes help to destroy the acini. Strands of dense fibrous tissue surround the individual acini. Type III: In the third reaction type, parts of the transplant resemble the second type; but in addition there are areas in which acini are closely packed together without being separated from each other by strands of fibrous tissue. At other places in the periphery there may be such bundles of acini with medium sized epithelium, in which the colloid is either softened and in process of absorption or in which the colloid has already been completely absorbed. Lymphocytic infiltration may cause the destruction of some of the epithelium. The center of the transplant consists of fibrous tissue in which there may be cell nests with squamous epithelium. These also are usually infiltrated with lymphocytes. In general, much lymphocytic infiltration is seen in the dense central fibrous tissue as well as in the remaining acinus tissue; the lymphocytes penetrate between as well as into the acini. In the parathyroid also there is usually lymphocytic infiltration. The lymph vessels are filled with lymphocytes. In some cases the lymphocytes very densely infiltrate the whole transplant.

Among tissues examined from twenty to twenty-five days after transplantation all three types are represented; but between the twenty-fifth and fortieth day the majority fall into Type I; only about one-third belong to Type II, in which the transplant is approaching complete destruction; while Type III is not found. In this series we had to deal with guinea-pigs which were either quite unrelated to one another or in which the relationship was at the most a very distant one.

In a second series of experiments in which nineteen transplantations were examined during the same period, the guinea-pigs used were received from the same city and from dealers who occasionally bought animals from each other. A relationship between the donor and host therefore could not be excluded with the same certainty as in the first series. In these animals, the results were on the whole similar, but slightly better. Of four transplants examined between the twentieth and twenty-fifth day, only one represents Type III. Of fifteen transplants examined between the twenty-fifth and fortieth day, six represent Type I; four, Type II; and in five the result is slightly better than in Type II without, however, reaching Type III.

In a third series we transplanted the thyroid into very young guinea-pigs, which were usually not older and sometimes were younger than ten days at the time of operation. The transplants were removed between twenty and twenty-five days following transplantation. The animals were believed not to be related to each other; but in this series we could not have the same degree of certainty in this respect as in the first series. In this third series the transplants are on the average somewhat better preserved. One case represents Type I; two, Types II and III; two, Type III; and in two other cases a still better preservation is obtained, approaching a condition which we find in syngenesiotransplantation. In one of these cases examined twenty days after transplantation, we find a ring of colloid containing acini. In the center of the ring there is at one pole some loose connective tissue with blood pigment cells; at the other pole the center is filled with dense fibrous tissue which also extends around the acini. There is much lymphocytic infiltration in the center as well as around the acini. In the second case, twenty-five days after transplantation, much thyroid tissue is left and the acini are close together; however the ring of thyroid tissue is not complete. The center consists of loose connective tissue together with some denser fibrous tissue; large vessels penetrate through the thyroid ring into the center. There is an intense lymphocytic infiltration in the center around the acini. Moreover, lymphocytes and probably also connective tissue cells invade and destroy acini. The parathyroid is also infiltrated with lymphocytes. There is no doubt that in the third series the average preservation of the transplants is better than in the first and second series; but whether this is due to a somewhat closer relationship between donor and host in some of these cases or whether the early age of the host delays the attack on the transplant by connective tissue and lymphocytes is not certain. However, in these very young animals the lymphocytic and connective tissue reaction is marked and we may assume that in all probability even in these cases ultimately the transplant would have been destroyed by the host. We may, therefore, conclude that even in transplantations in which we can with certainty exclude a near relationship between donor and host, small parts of thyroid may still be preserved as late as forty days after transplantation; and that between the twentieth and twenty-fifth day we may in some cases still find some clumps of well preserved

acini which are not separated by connective tissue from each other; but in both cases the connective tissue cells and lymphocytes continue the active destruction.

II. EARLIER PERIODS AFTER TRANSPLANTATION. In the foregoing experiments we determined the variations which occur in the homoiotransplants, at a time when the homoio-toxins have had a chance to exert their characteristic influence on the transplant. It was then of interest to determine how soon these variations are recognized in the earlier stages of transplantations and we examined therefore a considerable number of transplants between the seventh and twentieth day following the operation.

1. *From 7 to 10 days after transplantation.* During this period the center of the transplant still consists of necrotic material. We may find in this necrotic center a differentiation between a central part which contains pyknotic nuclei, and a peripheral part in which the nuclei are entirely dissolved. The acini are arranged in the form of a ring which is interrupted in certain places, especially where fat adjoins the acini. The fat interferes with the nourishment of the neighboring thyroid tissue and thus causes its necrosis. It is especially through interstices in the thyroid tissue that blood vessels grow into the center; but in homoiotransplants, these vessels are usually narrow, as already pointed out by Hesselberg, and the resulting vascularization of the center is therefore much inferior to that found in autotransplantation. Furthermore, the vascular, slightly edematous zone at the edge of the inner part of the ring, which is often found in autotransplantation, is lacking in homoiotransplants. In studying the ring of acini, it is found that some of the acini contain solid, retracted colloid, but that even in this early period there are many acini without colloid or with colloid in process of destruction. The destruction of the colloid is caused largely by mononuclear cells which are in the lumen of the acini. At this stage also connective tissue cells and lymphocytes may surround, invade and destroy acini. The connective tissue cells form fibrillar and fibrous tissue around the acini, and fibroblasts penetrate into the central necrotic material where they often degenerate again owing to the lack of proper nourishment. Notwithstanding these unfavorable conditions, mitotic proliferation still takes place in some of the acinus cells. The lymphocytes are not yet as numerous as at some later periods, but they are distinctly increased and they tend to

collect, especially at the inner part of the ring of thyroid tissue, and also to infiltrate the parathyroid; the lymph vessels surrounding the transplant show likewise an increased number of lymphocytes. Homoio-substances developed under these conditions attract and stimulate to activity the cells of the host tissue.

2. *From 11 to 12 days after transplantation.* At this period the difference which we observed at later periods between homoiotransplants in different individuals, is already noticeable. Again we carried out a series of transplantations into very young guinea-pigs, and another one into older animals weighing approximately 500 to 800 gm. In the former series, the homoio-reaction is already quite distinct at this period. The center is entirely organized by connective tissue in some instances, but in others there may still be found some unorganized, partly hemorrhagic material in the center of the transplant. The vessels growing into the center are, on the whole, narrow, although occasionally some vessels are larger. Fibroblasts and fibrous tissue may surround acini; lymphocytes, which accompany especially those vessels passing through the thyroid, likewise surround certain acini and perhaps penetrate into some of them. Thus in some places a destruction of acini may be observed at this period, but in other places the acini are still well preserved and lie close together and here the colloid is better preserved than in the areas where connective tissue and lymphocytes invade the tissue. There seems to be a distinct connection between the activity of the connective tissue cells and lymphocytes and the preservation of the colloid. In one specimen from a very young guinea-pig in this series the character is that of a syngenesiotransplant: the number of preserved acini is larger and the colloid is better preserved; in the center there is less dense connective tissue and the vessels here are wider, yet around the transplanted tissue there are noticeable collections of lymphocytes and in the adjoining fat tissue there is a slight epithelioid reaction.

In the second series in which the pieces had been transplanted into older guinea-pigs, the fibrous tissue and lymphocytic reaction is in principle the same as in young animals; but here the homoio-reaction is, on the whole, somewhat more marked, although certain gradations in the intensity of the homoio-reaction are observed. The fibrous tissue formation in the transplant and the lymphocytic invasions are generally more pronounced in these cases than in younger

hosts; furthermore we could not find in this series a specimen approaching the character of a syngenesiotransplant. Often great parts of the thyroid have been destroyed. However, we observed, even among the older guinea-pigs, areas in the transplant with well preserved acini lying close together and still containing good colloid; and occasionally a small, somewhat edematous area around a larger vessel is found in the periphery of the center. In later periods in a number of cases branching ducts with squamous epithelium are seen in the fibrous center; this epithelium has a greater tendency to mitotic division than the acinus cell. Lymphocytes invade also these ducts. We find then at this period already the same variations which occur at later periods and we note again, especially, that the homoio-reaction is less pronounced in the younger hosts.

3. *From 13 to 14 days after transplantation.* The findings were very similar to those of the preceding period. We observed the same differences between transplants which have been obtained from very young hosts and those from older hosts. In the former there occur again some specimens representing syngenesio-reaction or even approaching auto-reaction, in which many blood vessels enter the transplanted thyroid from the outside and penetrate toward the center where loose connective tissue surrounds the vessels and only a few lymphocytes are present; the acini in such cases are close together and contain good colloid. It is important that such reactions are observed even in cases in which host and donor were received from different dealers. On the other hand, we find in the transplants from young guinea-pigs also all the typical homoio-reactions in various grades of intensity. There is destruction of acini by lymphocytes and connective tissue, formation of fibrous tissue around acini and scant vascularization of the center. Already at this stage the center around which the thyroid ring is situated is much larger than in cases of auto- or syngenesiotransplantation. In the parathyroid also there is considerable lymphocytic infiltration. In older hosts again the homoio-reaction is more pronounced. In some instances we observe only scattered acini in the fibrous tissue and in others find only fibrous tissue. In those transplants in which a ring of acini is still preserved, the intensity of the lymphocytic and connective tissue reactions varies in different cases. Occasionally there can be found in the center of such transplants an area with

some large vessels which are surrounded by cellular connective tissue. Mitoses in acinus cells are quite frequent at this period.

4. *From 15 to 17 days after transplantation.* Conditions at this stage are approaching those found after the twentieth day. In pieces from young as well as older hosts Types II and III prevail. In older guinea-pigs there is perhaps a somewhat more marked lymphocytic reaction and more thyroid is destroyed, but on the whole the difference between these two kinds of transplants is not very great. Connective tissue and lymphocytic reactions are usually prominent in both series. As a result of the rupture of newly formed capillaries, hemorrhages occur in the center of the transplants although the vessels here are usually small. Such hemorrhagic necrotic material in the center is found incompletely organized as late as seventeen days after transplantation; especially in cases of localized infection, organization is distinctly delayed. At this period fibroblasts penetrate also into the central fibrous tissue and begin to replace it. Not only thyroid but also parathyroid tissue is invaded by lymphocytes and connective tissue; on the other hand, mitoses occur not only in the acinus cells of the thyroid, but also in the parathyroid cells. At the earlier periods the first type of homoiotransplant is found not quite as commonly as at later periods.

After homoiotransplantation of thyroid tissue into pregnant guinea-pigs, the same results were on the whole obtained as in non-pregnant guinea-pigs. However, in several cases we observed that after transplantation into guinea-pigs that are in an early stage of pregnancy the transplants are better preserved; the connective tissue and lymphocytic reactions, although present, are less pronounced than after transplantation into guinea-pigs in a more advanced stage of pregnancy.

Summary

1. If we follow the development of autotransplantation from early to late stages we notice a gradual elimination of those factors which distinguish an early transplant from the normal organ. Fibrous tissue, some fat tissue and very slight accumulations of lymphocytes gradually disappear; the fibrous tissue is at least in part absorbed through the activity of the fibroblasts acting in a satisfactorily vascularized zone. The tissue relations in the trans-

plant tend to attain the same mutual equilibrium which exists in normal organs. Mechanisms are called forth which eliminate temporarily disturbing factors and which disappear again after the disturbing factors have been eliminated. The normal equilibrium between tissues is determined by auto-substances which in this case are given off by acinus cells and which act upon the connective tissue cells, blood vessels and lymphocytes.

2. After homoiotransplantation of thyroid and parathyroid we find a certain range of variation in the behavior of the transplant which we determined quantitatively at different periods after transplantation. The large majority of homoiotransplants can be arranged in three types according to the severity of the destruction caused mainly by the activity of the connective tissue and lymphocytes of the host; and a certain kind of vascularization corresponds to this reaction. We find in the homoiotransplants variations ranging from complete destruction on the one hand to a condition approaching those found in syngenesiotransplants on the other. These variations between transplants begin to become noticeable as early as ten to twelve days following transplantation, and soon afterwards the characteristic difference in the behavior of the central parts, which exists between auto- and homoiotransplant, becomes distinct.

3. While these differences in the behavior of the transplants are essentially graded in accordance with the genetic relationship of donor and host, there are some indications that non-genetic variable factors may to a certain extent modify the intensity of the reaction. Thus it appears that while the homoio-reaction shows the typical characteristics in old as well as in very young hosts, it is less pronounced after transplantation into very young hosts than after transplantation into adult animals. How far these other variable factors (*e.g.*, pregnancy) affect the intensity of the homoio-reaction needs still further investigation.

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AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF CARTILAGE IN THE GUINEA-PIG*

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In previous investigations we analyzed the factors which determine the fate of transplants of various organs and tissues having a more or less specialized and complex character. We then thought it would be of interest to compare the fate of a relatively simple tissue like cartilage after auto- and homoiotransplantation with the behavior of the organs and tissues we had studied previously. Cartilage has already been used by a number of investigators in transplantation experiments. These earlier investigations were, however, mostly undertaken from viewpoints which differ from ours. Marchand [1] and Borst [2] have reviewed the older literature on this subject.

I. AUTOTRANSPLANTATION OF CARTILAGE

In the majority of cases, xiphoid cartilage with attached perichondrium, fat and areolar connective tissue and in some cases ear cartilage with attached particles of epidermis, hair follicles and fat tissue were used. In all experiments, the tissues were transplanted into subcutaneous pockets. The pieces were taken out for microscopic examination at periods varying from one day to five months nineteen days. Altogether thirty-two autotransplants were studied.

The results may be summarized as follows: After autotransplantations, cartilage and perichondrium may remain preserved *in toto* or parts of it may become necrotic. The latter effect is merely the result of accidental injuries due to the process of transplantation, namely, pressure exerted during the operation, lack of nourishment, hemorrhage in the tissue adjoining the transplant or localized infection. The surrounding areolar or fat tissue may often show in the first few days small localized necroses, some polymorphonuclear leucocytes here and there or hemorrhagic areas especially in the

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region of necrotic tissue. Near these hemorrhagic areas and in the fat tissue some leucocytes may be found in this period. Fibroblasts and capillaries may grow into the blood clots. Furthermore, connective tissue penetrates in some cases into wounds or necrotic areas in the cartilage. Fibroblasts also collect in the first week around the periphery of the transplant. Thus a slight increase in connective tissue can be found at various points in the transplanted tissue during the first week or so. At this time also, very small collections of lymphocytes can occasionally be seen especially around some vessels, around the perichondrium or elsewhere. But at a later period, we usually find the cartilage surrounded by a layer of loose connective and fat tissue which is almost free from lymphocytes and does not show any noticeable fibrous thickening. Also the polymorphonuclear leucocytes have disappeared. A partial or complete recovery may take place from the injuries received during and soon after transplantation. In autotransplantation usually no epithelioid or giant cell formation is found in the surrounding fat tissue, but occasionally such a reaction does occur. Around areas of necrotic cartilage, the perichondrium can proliferate and apparently replace the defects in the cartilage. There is, however, one reservation necessary in regard to this statement. Even in the normal xiphoid cartilage there is, in places where the cartilage is thicker, a shrinking and necrosis or solution of those parts of the cartilage which are further removed from the nutrient fluid and oxygen of the surrounding tissue. Around such necrotic areas of cartilage, regenerative new formation of cartilage on the part of the perichondrium may take place. It is therefore not always possible to decide how much of the perichondrial regeneration has taken place before and how much after transplantation.

Striated muscle tissue attached to the cartilage and in some cases transplanted with it, may be found well preserved five and one-half months after transplantation. In cases in which ear cartilage was transplanted, the attached epidermis formed cysts in the manner described by us previously.[3] Also hair follicles and sebaceous glands may be preserved. Thus the transplant with all its constituent parts can remain alive after autotransplantation. We must, however, state, as we have noted earlier in papers, that even in autotransplantation the epidermal cysts are sometimes a source of irritation. Some lymphocytes may collect around them, the host

connective tissue may gradually penetrate into them, and in this way the cyst may be slowly destroyed.

To summarize, we find after autotransplantation of cartilage into the subcutaneous tissue, the cartilage with the surrounding areolar connective tissue and fat tissue preserved throughout the whole period during which we examined it. Abnormalities which occur in the first period following transplantation are gradually eliminated.

II. Homoiotransplantation of Cartilage

Approximately seventy pieces of homoiotransplants were examined at periods varying between one day and five months nineteen days after operation. The cartilage was as usual placed in pockets in the subcutaneous tissue. After transplantation, cartilage and perichondrium may remain preserved *in toto*, or certain parts may become necrotic due to injuries of various kinds. As in cases of autotransplantation, hemorrhages may occur in the connective tissue surrounding the perichondrium. In the first few days, some collections of polymorphonuclear leucocytes may be found around the transplanted fat tissue; they may also penetrate into the fat tissue and even in places reach the perichondrium. Scattered leucocytes may also be found in the muscle fibers which after transplantation have become hyaline. However, this leucocytic infiltration varies in different cases and is usually very moderate. Under ordinary conditions, it disappears within the first week. Already within the first six days, connective tissue may begin to proliferate around the cartilage; it may invade the necrotic areas or separate perichondrium from the surrounding areolar fat tissue. When hemorrhages are present, fibroblasts and capillaries penetrate the clot. The transplanted muscle tissue soon becomes necrotic. Connective tissue cells invade also the fat tissue and may separate fat cells from each other. Toward the end of the first and in the beginning of the second week some larger mononuclear cells may appear in the fat tissue. At this period there are also occasionally noticeable here and there some slight vacuolization and other changes of a degenerative character in the intercellular substance of the cartilage; but on the whole, the cartilage remains preserved and one may even observe some perichondrial regeneration and beginning new formation of cartilage. Lymphocytic infiltration becomes definite at this stage,

although it is as yet slight. Lymphocytes appear first in the transplanted fat tissue and in the loose connective tissue around the perichondrium.

In the course of the second and third week after transplantation, these changes gradually develop still further. The lymphocytes increase in number, but the intensity of the lymphocytic infiltration is variable, being on the whole moderate, though it may be more marked in certain animals. It varies also in different places. There is a tendency for the lymphocytes to collect especially around the perichondrium and they may even penetrate into it, but they are found also in the fat tissue and particularly in the fibrous tissue strands. In rare cases toward the end of the third week, the lymphocytic infiltration may become so strong that the cartilage becomes sequestered. The epidermal cyst which usually forms after transplantation of cartilage to which some skin is adherent, may be markedly infiltrated and even destroyed by lymphocytes; but when there is dense fibrous tissue around the epidermis, this may protect the epidermis against their onslaught. Connective tissue proliferation continues. Either the connective tissue septa in the fat tissue may become moderately fibrous or the greater part of the fat and areolar tissue may gradually be replaced by fibrous tissue. Fibroblasts usually continue to penetrate the necrotic areas of cartilage and a dense layer of fibrous tissue gradually develops around the cartilage; but in other cases, a considerable amount of areolar and fat tissue may remain preserved around the perichondrium. In the second week, epithelioid and giant cell formation can be fairly often observed in the fat tissue. Proliferation of the perichondrium takes place at this period, but it is remarkable how rarely mitoses seem to be found in this tissue. We have seen only one definite mitosis in a perichondrial cell.

Again we must keep in mind that even in the normal untransplanted xiphoid cartilage, a partial necrosis may occur in the parts which are thicker and that at such points perichondrial cartilage may normally replace the necrotic material.

During the fourth week, the reaction of the host tissue to the transplant is fully developed. The cartilage and perichondrium are usually well preserved, but some necrotic areas may occur in the cartilage, for instance, at the cut end. Also in the center of thick cartilage, where nourishment is less favorable, cartilage cells may

shrink and there may be in places a solution of cartilage substance. Perichondrium produces especially around necrotic cartilage, new cartilage with typical intercellular substance. Furthermore, connective tissue with lymphocytes and sometimes with blood capillaries may penetrate the necrotic area and replace it. The perichondrium is usually well preserved, but it is often infiltrated with lymphocytes which occasionally destroy great parts of it. On the other hand, lymphocytes penetrate the hyaline cartilage not at all or only for a very short distance. They do not destroy healthy cartilage to any large extent. However, at points where the cartilage is cellular and where the hyaline intercellular substance is largely lacking, the lymphocytes invade the cartilage often over a considerable area and succeed in destroying at least a part of it. On the whole, the lymphocytic infiltration is moderate in the transplant. It is found especially around the living cartilage where dense fibrous tissue surrounds cartilage or perichondrium. It may also be observed in the fat or areolar tissue when they are still preserved in the transplant. In places where the lymphocytic infiltration is more marked, the number of lymphocytes in the lymph vessels is also much greater. The connective tissue around the cartilage and perichondrium is increased and the amount of preserved fat and areolar tissue is decreased. In many cases, the fat tissue is entirely replaced by fibrous tissue and when the fat or areolar tissue is preserved, the septa often show fibrous thickening. Frequently we find in the remaining fat tissue epithelioid and giant cell reaction. In one specimen, material resembling bone was found and it had apparently originated in a place where the cartilage had become necrotic.

In the transplants of ear cartilage, conditions are on the whole similar. Lymphocytes are observed to penetrate necrotic parts of the cartilage. The epidermal cyst which has formed at a previous period may be destroyed at this time and only keratin may be found, around which epithelioid and giant cells have developed. In some specimens, epidermis, connective tissue and perichondrium show intense lymphocytic infiltration.

During the second month, conditions remain about the same as in the preceding period as far as the behavior of cartilage, lymphocytes and connective tissue is concerned. In different cases the amount of fibrous tissue produced and the amount of fat and areolar connective tissue preserved vary, but on the whole the fibrous tissue

formation and substitution of dense fibrous tissue for loose areolar and fat tissue are very pronounced at this period. However, there are often areas of the latter tissue still left and in the preserved fat tissue an epithelioid and giant cell reaction quite commonly occurs. The amount of lymphocytic infiltration also is variable; it is usually most marked around living healthy cartilage and in places where the connective tissue surrounding the perichondrium is dense. The lymphocytes occasionally penetrate a little the periphery of the hyaline cartilage. They move lengthwise here in the direction of fibrillation in the peripheral cartilage and gradually they perish owing to the pressure exerted upon them and to the lack of nourishment. But this injurious action on the part of the lymphocytes makes it occasionally possible for connective tissue cells and blood capillaries to penetrate for a short distance the marginal part of the cartilage. Otherwise they penetrate only necrotic cartilage. In the tissue surrounding the cartilage, lymphocytes often accompany the blood capillaries and are found accumulated in the lymph vessels. The capillaries in the transplant are in some cases directed vertically toward the cartilage. When they have nearly reached the cartilage, they turn in the opposite direction. However, at the turning point they are liable to be injured and to give rise to hemorrhages.

In three animals the homoiotransplanted cartilage was examined five months and nineteen days after operation. It is of interest to note that in these cases the reaction of the host against the transplant is not more intense than in the earlier periods. On the contrary, over wide areas the lymphocytic reaction is relatively mild and in these cases fat tissue as well as areolar tissue is preserved. However, at this late period it is observed in one specimen that blood capillaries, connective tissue and lymphocytes penetrate areas of hyaline cartilage where the cartilage cells are widely separated or have perished owing to the deposition of a large amount of hyaline material. Presumably through the penetration of the body fluids of the host into this material, it had assumed a very soft character. Nevertheless, as we stated, on the whole the destruction of cartilage is slight even at this late period, although areas of necrotic cartilage can still be found.

Homoiotransplantation of Bone Marrow

In several cases bone and bone marrow adjoining the xiphoid cartilage were homoiotransplanted together with the cartilage. The examination took place between twenty and thirty-five days following transplantation. In every case it is found that the bone marrow has been replaced either by myxoid connective or by fibrillar connective tissue. There are also lymphocytes in this connective tissue. The bone itself is surrounded by some foreign body giant cells and the zone of proliferating cartilage normally adjoining the bone has become necrotic.

Discussion

If we compare autotransplantation of cartilage with autotransplantation of the thyroid gland in guinea-pigs, we observe an essential similarity in both. After temporary injuries and consequent reactions on the part of the host, the transplant recovers. There may be some necrotic parts of the cartilage which are replaced by connective tissue or gradually by regenerated cartilage produced by the perichondrium; but on the whole, cartilage, loose areolar connective tissue and fat tissue return to their previous condition. After some time the tissue has assumed the character of normal tissue. Small numbers of lymphocytes and slight amounts of connective tissue which may have been seen at first are no longer in evidence at later stages. Epithelioid and giant cell formation in the fat tissue occurs less often in the auto- than in the homoiotransplant. Even well preserved striated muscle tissue may be noticeable in the autotransplant at later periods. The same forces that cause the old equilibrium to be reëstablished in the case of thyroid, are equally successful in the case of cartilage and fat tissue transplants.

A similar parallelism exists in principle between homoiotransplants of thyroid and cartilage. In both tissues there may not be at first a marked difference in the state of preservation from that of the respective autotransplants; but in both kinds of tissue the activities of the connective tissue and of the lymphocytes soon lead to a great difference between the auto- and homoiotransplants. Even the time following transplantation, when these activities on the part of the connective tissue and lymphocytes set in, is the same in both instances. In the case of the thyroid, the fibroblasts form

dense connective tissue in the center as well as around the transplant. In the case of the cartilage and fat transplant, the connective tissue penetrates between the fat cells and produces fibrous septa between them; or it may entirely, or at least over considerable areas, replace the fat and loose areolar tissue. It may surround the cartilage and penetrate into the necrotic cartilage, but it does not under normal conditions penetrate into the living cartilage. As far as the connective tissue activity is concerned, the transplanted fat tissue behaves, therefore, essentially the same as transplanted thyroid, while the reaction of transplanted cartilage is quite different.

In regard to the lymphocytes, it is seen that they invade and help to injure the strange tissue in homoiotransplants of both thyroid and cartilage. The lymph vessels are filled, and the lymphocytes appear in increased numbers in the fat and loose areolar tissue. They form also after some time a layer around the perichondrium, which is, however, usually not complete and varies much in density in different animals. If we compare in the same animal homoiotransplants of the thyroid and of the cartilage, we find that there is in general a parellelism between them. In those animals in which the thyroid transplant shows the densest lymphocytic infiltration, there we also find in the cartilage and fat tissue transplant relatively the most marked lymphocytic infiltration. But absolutely there are decided differences between the reactions in the various kinds of tissues. In the first place, the lymphocytic infiltration is almost invariably less marked, and sometimes considerably so, in and around the cartilage and fat transplants than in and around the thyroid transplant. Furthermore, in the latter the lymphocytes have no difficulty in penetrating between and into the acini, thus ultimately destroying them. The cartilage, on the other hand, is very resistant to attack on the part of the lymphocytes, though the latter may invade the perichondrium and succeed in entering for a very short distance the periphery of the cartilage proper. At times, however, although relatively rarely, one may even see lymphocytes press themselves into the cartilage substance, following the path of least resistance; but here they are subjected to considerable pressure on the part of the hard cartilage substance, their nuclei often assuming unusual shapes, and soon the death of the cells occurs. Thus it is seen that lymphocytes do not succeed usually in penetrating far into cartilage. How-

ever, in the small area where they occasionally penetrate, they may pave the way for the entrance of connective tissue and blood capillaries for a limited distance into the periphery of the cartilage.

On the other hand, cartilage homoiotransplants may be invaded by lymphocytes under certain conditions, namely: (1) Lymphocytes may accompany connective tissue and blood vessels in their invasion of necrotic cartilage. (2) Lymphocytes may invade cartilage which consists mainly of cartilage cells and in which only very little intercellular hyaline cartilage substance has been produced. Such cartilage may be found at the end of the transplant corresponding to the cellular cartilage which is normally found at the end of the xiphoid cartilage. Lymphocytes may invade this cellular cartilage tissue in such large numbers that in some cases they may almost completely destroy it. (3) At a late stage degenerative processes may at times occur in the cartilage which lead to a broadening and apparently a softening of the cartilage substance. The lymphocytes may invade such softened areas. These are additional proofs of the destructive power and function which the lymphocytes possess and may exhibit in cases of homoiotransplantation.

We see then that the cartilage transplant resists the aggressive and destructive activity on the part of the lymphocytes mainly on account of its mechanical property of hardness. Thus it comes about that the homoiotransplanted cartilage remains preserved, at least for very long periods of time, while the large majority of other tissue transplants are quite commonly destroyed by lymphocytes and connective tissue. This applies also to the fat tissue transplanted with the cartilage, which is destroyed to a much larger extent than the cartilage. There is, however, still a second factor which tends to exert a favorable effect on homoiotransplants of cartilage and fat tissue. This is the fact that these tissues, even where they are soft, cause in many cases a less pronounced attraction of the lymphocytes than does the thyroid. As to the reason for this difference we cannot be quite certain. It may be due to a less active metabolism on the part of such tissues. A smaller quantity of metabolic products is given off which attract the lymphocytes. Or, perhaps, it may be due to the less active growth of lymph vessels into the transplant of fat, areolar and cartilage tissue as compared with the growth into thyroid. We often notice such differences in other organs. Thus the

homoiotransplants of the parathyroid are often somewhat less actively invaded by lymphocytes than the adjoining thyroid. However, lymphocytes do invade and destroy also parathyroid tissue fairly actively. That a diminution in the formation of chemotropically active substances play a rôle in this condition is made probable by the fact that the lymphatics which are noticeable in the transplant are usually less crowded with lymphocytes in the neighborhood of the cartilage than in the thyroid transplant.

Another observation of interest which we made concerns the absence of cumulative action on the part of the cartilage transplant on the lymphocytes. The lymphocytic infiltration does not increase following the first three weeks after transplantation. On the contrary it appears as though a decrease in the intensity of lymphocytic infiltration may take place in the course of time.

We see that in principle the same factors play a part in transplantation of cartilage and fat tissue as in transplantation of thyroid, but that certain quantitative differences in the intensity and effectiveness of these factors exist in these cases. That the cartilage and perichondrium as such are not injured to any marked extent by the body fluids of the host after homoiotransplantation is also evident from the fact that the perichondrium may regenerate and form cartilage in the case of homoiotransplantation as well as of autotransplantation. As we stated, mitotic proliferation may play a part in this process, but it seems to be of an extremely low degree of intensity. This regeneration occurs especially around areas of necrotic cartilage, and we have seen that similar degenerative processes and subsequent perichondrial regeneration may occur normally in the xiphoid cartilage in places where its diameter is greater and where therefore the tissue is less well supplied with food and oxygen.

Summary

1. After autotransplantation, cartilage, perichondrium, fat, areolar connective tissue and sometimes also striated muscle tissue remain well preserved. After homoiotransplantation, cartilage also remains on the whole well preserved. Perichondrium may form new cartilage in both instances. On the other hand, areolar and fat tissue after homoiotransplantation are usually invaded and partly or completely replaced by connective tissue, and the epithelioid and

giant cell reaction in the fat tissue seems to be much more frequent than after autotransplantation. Bone marrow also after homoiotransplantation becomes more or less replaced by connective tissue.

2. After autotransplantation, lymphocytes are lacking almost entirely in the cartilage transplant. After homoiotransplantation, lymphocytes collect first in the fat and loose connective tissue and then penetrate toward perichondrium and cartilage. The reaction begins early, reaches a maximum about three weeks after transplantation and from this time on ceases to increase to any great extent. In different cases and at different points of the transplant, the lymphocytic reaction varies considerably. On the whole, the reaction is much less pronounced in the cartilage than in the thyroid transplant; this may in part be due to the lower intensity of metabolism in the cartilage. Therefore, both lymphocytes and connective tissue play in principle the same rôle as agencies of attack in the homoiotransplant of cartilage and adjoining tissue as in other tissues, although on the whole the reaction is weaker in the former than in the latter kind of transplants.

3. The lymphocytes as well as connective tissue and blood vessels may readily enter necrotic areas of cartilage and replace them, but they can migrate only to a very limited extent into living, well differentiated cartilage and soon they perish there. Occasionally lymphocytes and also connective tissue may penetrate somewhat deeper the hyaline cartilage substance, when the latter is somewhat softened. On the other hand, lymphocytes find their way readily into cartilage when it is still composed mainly of cartilage cells and when the cells are separated by only a very slight amount of intercellular substance. This cellular cartilage can be as successfully destroyed by lymphocytes as other soft tissues.

4. The great power of survival of cartilage after homoiotransplantation is, therefore, due to two factors: (1) a diminution in the intensity of the homoio-toxic action, and (2) to a greater resistance of the cartilage toward the instruments of attack on the part of the host, namely, the lymphocytes and connective tissue cells. The individuality-differential is developed in cartilage as well as in other tissues. The greater power of resistance of cartilage depends upon the formation of the hyaline intercellular substance. Whenever this is much decreased in amount, the cartilage falls as easy a prey to the lymphocytes as do softer organs and tissues.

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SECONDARY GLIOMATOSIS OF THE LEPTOMENINGES *

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In 1924, Ford and Firor [1] published, with a review of the literature, four cases of primary sarcoma of the leptomeninges, one of which later proved to be a glioma. The following month, they [2] reported another case of secondary gliomatosis of the leptomeninges making a total of ten cases. They also reviewed and abstracted the available literature on this subject.

Since the publication of the papers of Firor and Ford, another case has been reported by two Dutch authors.[3] The writer has fortunately been able to locate a few other examples of this peculiar and interesting disease, which were not included by Firor and Ford. Since Mallory's [4] and Strümpell's [5] gliomas are not indexed it was only by good fortune that they were discovered. These cases are briefly abstracted and bring the total number of such up to eighteen, including the one reported below. Abstracts of all the cases of secondary gliomatosis of the pia-arachnoid are now available in English. As many authors state, and as occurred in one of the cases of Ford and Firor, it is not always easy to differentiate between glioma and sarcoma of the meninges and probably many tumors of the latter belong to the former group. The tumor described by Kooy [6] is added to emphasize this point, while the primary growth reported by Stern [7] was a glioma but the meningeal changes were incompletely studied. Both of these cases appear to be doubtful. The technic described by Mallory [4] in his paper referred to below, and in Mallory and Wright's [8] book, should always be used.

LITERATURE

It appears that Strümpell [5] in 1897 was perhaps the first to call attention to this unusual disease. He demonstrated a case in a medical meeting, but the details are lacking.

In Mallory's [4] admirable paper which appeared in 1908, on the application of the phosphotungstic acid stain, he briefly refers to a glioma of this group (page

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582). A large glioma was found involving the lumbar spinal medulla of a child. Much of the cord had been destroyed, and the subarachnoid space was filled with abundant tumor growth which had also extended upward about the base of the cerebellum and cerebrum both of which were invaded. At the necropsy table, the meningeal growth was regarded as an unusual meningitis. The tumor cells consisted mostly of large spindle and spherical cells while multinucleated giant cells were also observed. Glial fibrils were demonstrated.

In 1912, Mees [9] described a glioma of the spinal cord in a man 43 years of age. The symptoms were those of paresthesia and spastic paralysis. A clinical diagnosis of syphilitic meningitis was made. At necropsy there was found a very extensive glial growth which had arisen in the cervical cord, almost surrounding it, and extended about the medulla oblongata and pons. Under one temporal lobe was a small metastasis. The tumor cells were small and of various sizes and shapes but giant cells were not a feature. Glial fibrils were abundant. Certain areas of the meningeal growth were very cellular. Apparently, the extension in this case was by way of the leptomeninges, but Mees has made no special point of it.

Löwenberg's [10] cases appeared in 1921. The first one was in a child 12 years of age with a symptomatology indicating an intracranial growth. A large reddish and degenerating tumor of the optic thalamus was found postmortem, which had invaded the third ventricle. The leptomeninges of the base of the brain, cerebellum and cord were thickened by the infiltrating glioma, and the growth was abundant about the corpora quadrigemina. The spinal cord was considerably distorted by the tumor growth. The tumor tissue also followed along the nerves. The neoplastic cells were variable and some were multinucleated. Glial fibrils were present.

The second tumor reported by Löwenberg was found in a child 11 months of age with the clinical evidences of a brain tumor. A growth of the optic thalamus was found which had invaded the choroid plexus and the ventricular walls, where it formed a papillary growth. The lateral ventricles were dilated. The tumor apparently extended backward and involved the leptomeninges of the cerebellum and cerebrum. Microscopically, the glioma was composed of various cell forms and glial fibrils, but giant cells were absent. The spinal cord was not examined.

The case reported in 1925 by deLange and Smid [3] was a boy 2 years of age. The child presented diffuse and progressive neurologic findings which early in the disease were regarded as those of tuberculous meningitis. The cells in the cerebrospinal fluid were increased in number and the globulin test was positive. At necropsy, on the base of the brain and especially about the optic chiasm there was a soft mass in the subarachnoid space, which was regarded as a peculiar meningitis. The tumor apparently was primary in the basal ganglia. The ventricles were dilated. There was growth about the cranial nerves, but the spinal cord was not examined. They describe the tumor cells lying in a network of glial fibrils.

Stern [7] recorded a glioma of the fourth ventricle in a boy 15 years of age. The tumor had occluded the median foramen of the fourth ventricle and had involved the choroid plexus. Clinically, the patient presented symptoms of a brain tumor. Stern describes what he regarded as a chronic meningitis of the cerebellum and cerebrum, but he made no microscopic study of the meningeal lesions. The choroid plexus was also invaded by the tumor. He undoubtedly

was dealing with an extension of the ventricular tumor, but the case will remain doubtful because of the incomplete examination.

Kooy's [6] case which he described as a sarcoma or perhaps an endothelioma, had characteristic features of a diffuse secondary gliomatosis of the pia-arachnoid. The primary tumor was a large solid growth in the cerebellum. The phosphotungstic acid hematoxylin stain of Mallory was not used and his diagnosis is certainly open to question.

Case Report

Clinical History: Dorothy M., a white girl 13 years of age, was admitted to the Allegheny General Hospital, Feb. 20, 1925, and died Feb. 25, 1925, following a craniotomy. She was brought to the hospital in a stuporous condition and a meager history was obtained from the family. The chief complaints were headache and vomiting.

The family and past histories were unimportant aside from the fact that the menstrual periods had not yet begun.

The illness probably began during the preceding summer, with frontal headaches which recurred with severity during the fall. In the meantime, she had had trouble with her eyes, and glasses which had been obtained apparently gave some relief. Six weeks previous to admission the frontal headaches and vomiting became severe. The vomiting was frequent and unassociated with nausea. The headaches persisted. Eight days previous to admission there had been a generalized convulsion accompanied, it seemed, by loss of consciousness. A similar convulsion occurred the following day; each time these paroxysms lasted about half an hour. During the few days following the convulsions, she became lethargic and was brought to the hospital in that condition.

Physical Examination: The patient was described as a well developed white girl who was drowsy but easily aroused. The cranium was negative. The right eye was slightly forward. There was a definite anisocoria, the right pupil being the larger. Both pupils were regular and reacted sluggishly to light and during accommodation. There was no nystagmus. The left nerve head was elevated four diopters, and the right five diopters. No retinal hemorrhages were seen. Otherwise the head was negative. The thorax was not remarkable and the heart and lungs presented nothing of importance. The abdomen also was negative.

The knee and triceps reflexes were diminished, while the biceps and abdominal reflexes were absent. The ankle jerks were present

though diminished. Plantar stimulation gave a normal response. There was some rigidity of the neck. Finger-pointing tests were poorly done. Adiadochocinesia was slightly positive in the right upper extremity.

Course: During the five-day course in the hospital before her death, four lumbar punctures were made, which greatly relieved the severe headaches. The cerebrospinal fluid was under marked pressure. It was clear and sparkling. The cell count made on but one occasion, revealed sixteen cells per c.mm. The globulin test was positive and the Wassermann test negative. An X-ray of the cranium failed to show any evidence of increased intracranial pressure or tumor.

The vomiting persisted and was projectile in character. On the 23rd of February the patient had a generalized convulsion, but the details are not available. The neurological findings remained essentially the same. By the 25th of February she had become totally unconscious. Without an anesthetic a left temporal decompression was performed, immediately following which the patient died.

Aside from an occasional slight elevation to 99 to 99.4 F, the temperature was essentially within normal limits until a terminal rise of 100.6 to 101.6 F. The pulse rate was very irregular but generally accelerated throughout, ranging from 66 to 120 per minute, and averaging about 110 per minute. The respiration rate was not remarkable aside from a terminal irregularity.

The urinalyses were negative. Red blood cells 5,312,000 and white blood cells 9,900 per c.mm.; hemoglobin 95 per cent (Sahli).

Clinical Diagnosis: Brain tumor, unlocalized.

Necropsy

The postmortem examination was performed by the writer, one and one-half hours after death.

The body was that of a poorly nourished white girl. It was essentially negative externally, aside from the evidence of a recent left craniotomy. The incision extended backward 12 cm. from the hair line in front and 2 to 3 cm. above the left ear. The right eyeball was a little more prominent than the left.

Upon stripping back the scalp, a recent oval surgical defect measuring 2.5 × 3 cm. was found in the left temporal bone. The exposed

dura was bulging and had a linear incision 2 cm. in length, through which the gray cortex could be seen. The skull was generally thinned.

Brain: The brain was normal in shape, but the convolutions were uniformly and markedly flattened. The organ weighed 1115 gm.

Changes about the base of the brain were indicative of a chronic meningitis. Here the leptomeninges were dull and opaque. The subarachnoid space and cisterna were filled with what was taken to be a thick exudate. This material obscured the large vessels of the base of the brain as well as the optic chiasm. The cranial nerves could not be followed. The material seemed to be limited primarily to the base of the brain, but also extended out to the tips and to a slight extent on the lateral and anterior aspects of the temporal lobes. The base of the cerebellum also was partially covered by the gray material, which likewise covered the under surfaces of the medulla oblongata. A large and fresh meningeal hemorrhage measuring 6 × 8 mm. was found lying just anterior to the pons. Other occasional and smaller hemorrhages were also seen. Otherwise the leptomeninges over the convexities were smooth and glistening. The cerebrospinal fluid in the subarachnoid space was greatly reduced and the brain was rather dry. Certain of the large vessels were dilated but otherwise externally the brain was negative.

It should be mentioned that in removing the brain the floor of the third ventricle was accidently torn, thus liberating a large quantity of clear fluid.

Upon splitting the corpus callosum longitudinally, a large and firm tumor was found. It projected from the left basal ganglia and was slightly yellowish in color. The mass extended well to the right of the midline and displaced the right basal ganglia. The tumor appeared to involve primarily the anterior and upper portions of the optic thalamus. The corpus striatum was enlarged and the pineal body was adherent to the tumor. The neoplasm projected 1 to 1.5 cm. to the right of the midline and measured 3 cm. in width and 4 to 5 cm. in length. In general, the surfaces of the tumor were smooth, but where it formed the walls of the third and lateral ventricles, the surfaces were coarsely granular.

On sectioning the tumor at right angles to its long axis, it showed a slightly yellowish tint in the thalamic portion especially medially, but laterally it gradually merged with the brain tissue. The texture was fine and there were no hemorrhages or other obvious degenera-

tive changes. There was no gross evidence that the tumor had invaded the floor of the third ventricle.

The upper portion of the third ventricle appeared to have been obliterated, while the thin-walled lower part which was ruptured in removing the brain, certainly had been dilated. The orifice of the cerebral aqueduct was flattened and apparently partially occluded. Both lateral ventricles were moderately dilated and contained clear fluid. The interventricular foramina presented nothing remarkable. The choroid plexus showed no noticeable abnormality. The ventricular surfaces were otherwise smooth. On section, the cerebral aqueduct and the fourth ventricle and its apertures were not abnormal.

The cerebellum presented nothing pathologic.

From the cut surfaces of the cross-sections of the brain stem and base of the brain, the soft white material regarded as exudate was found sharply limited to the subarachnoid space.

The dura was everywhere smooth and glistening and presented nothing abnormal.

The hypophysis grossly was not abnormal.

Spinal Medulla: Only the upper half-dozen segments of the spinal medulla were removed. Exactly the same condition was found in the leptomeninges of the cord as described over the brain, but the thickening was less marked. Otherwise the cord was negative in gross.

Heart: The right chambers of the heart were somewhat dilated, but otherwise the cardiovascular system was negative.

Lungs: The left lung was densely adherent and the pleura was thickened. A few calcified subpleural tubercles in the upper lobe and similar small tubercles in the lymph nodes of the hilum were observed.

The right lung was adherent about the apex, but it was otherwise negative.

Genitalia: The vagina and uterus were double and bilaterally symmetrical. The vaginal septum extended from the external orifices upward and between the two cervixes. Each uterus was approximately the size of one's little finger and curved laterally. The uterine tubes and ovaries were small but their relationship was essentially normal.

The abdominal viscera, aside from the alterations of the internal genitalia, were not unusual.

Cultures: From the craniotomy incision, meninges and heart's blood, *Staphylococcus albus* was obtained. The presence of colon bacilli in the culture of the blood and diphtheroids from the meninges were regarded as of no importance.

Microscopic Examination

Fixation was by Zenker's fluid; the stains employed were eosin-methylene blue, phosphotungstic acid hematoxylin and Mallory's aniline blue stain.

Tumor of the Basal Ganglia: The growth extends throughout the greater portion of the left basal ganglia. It is a glioma, though not altogether typical.

In the sections of the projecting and slightly yellowish portion of the primary tumor, the appearance is quite characteristic of a glioma (Fig. 1). The cells are irregular in size, generally fusiform and stellate in shape and lying in a fine network of fibrils. The cells are fairly numerous and nowhere are they closely packed. The cell bodies are mostly occupied by an oval or an elongated nucleus which contains a good deal of chromatin. Certain of the nuclei are more or less vesicular, especially in the larger and giant cells. The cytoplasm is not abundant but deeply basophilic. Extending from the irregular side of the cells are fine processes, which are lost among the fibrillar network. Mitotic figures are not numerous but certain fields contain two or more. Tumor giant cells, most of which are multinucleated, are numerous in places. Certain cell clusters resemble giant cells and some are impossible to differentiate. Fine cytoplasmic processes are also given off from the giant cells.

In the deeper portions of the growth it is very difficult to find any line of demarcation between the infiltrating tumor growth and the more normal brain tissue. Here one finds cells which are undoubtedly well preserved normal nerve cells still remaining in the extensively invaded tissue.

Where the neoplasm forms the walls of the ventricles, the structure of the tumor and character of the cells are somewhat different. The granulations described above prove to be small superficial areas of very compact but much more cellular tissue. The base of the choroid plexus which stretches over the tumor, is extensively invaded. The infiltrating growth of the choroid plexus is composed of

pale staining and coarse tumor tissue. The cells in these areas are large and mostly spindle-shaped, but not numerous. Fibrils here are not easily identified. The epithelial covering of the plexus remains intact and there are very few adhesions. Tumor giant cells are more numerous in the superficial tissue near the ventricular surfaces than elsewhere.

Lying in the midst of the loose tumor mass is a small round area of a quite different structure (Figs. 5 and 6). This area to the naked eye is sharply defined and measures 4 mm. in diameter. It is composed of narrow interlacing bands and strands of large fusiform cells. A fair number of these cells are in mitosis and occasional giant cells are observed. At the edges there is no very clear transition between the delicate glial tissue and the coarser bands of the nodule. A very little intercellular substance is present. Fibrils in this portion of the neoplasm are not found.

The blood vessels of the tumor are small, poorly formed and not numerous. Only an occasional focus of degeneration is found and wandering cells are sparse. There are no hemorrhages.

The fine fibrillar network of the neoplasm takes a deep blue stain with phosphotungstic acid hematoxylin. Delicate neuroglia fibrils are very numerous and sharply defined. In the denser portions of the tumor of the ventricular walls, fibrils are not numerous, though definitely identified. No fibrils are found by the aid of the special stain in the peculiar small and well-circumscribed area described. The intercellular fibers here take a yellowish brown color suggestive of collagen fibers. The cells are stained blue. Mallory's aniline blue connective tissue stain applied to the sections with the small nodule, gives a diffuse red color to the cells and to the scanty intercellular substance as well.

Brain Stem and Meninges: The sections of the base of the brain and brain stem together with the leptomeninges, reveal a peculiar neoplasm filling the subarachnoid space. There is no evidence of meningitis. The tumor is sharply limited externally by the arachnoid which in places is slightly thickened. Nowhere has the neoplasm invaded the brain tissue. Growth about the vessels which penetrate the brain cannot be demonstrated; however, many of the vessels of the meninges are covered by a thick mantle of tumor tissue. The cranial nerves are surrounded and embedded in this growth. Invasion of the nerves is absent.

About the optic chiasm one finds a very delicate glial tissue which is not unlike the structure of the tumor of the basal ganglia (Fig. 2). Numerous irregular tumor cells similar to those described are lying in a network of fibrils. Laterally, however, the growth becomes more cellular and compact and resembles more or less the localized thickenings of the ventricular walls. Elsewhere in the meninges, especially about the brain stem (Fig. 3) and also under the temporal lobes (Fig. 4) the growth is much coarser in structure. In places there are more or less defined transition zones from the more delicate to the coarser growth. Numerous tiny whorls are in the meninges composed of only a few tumor cells and with much intercellular substance, suggestive of collagen. The appearance somewhat suggests the structure of the endotheliomas of the meninges. Still other small whorl-like masses are found composed of numerous spindle-shaped cells with but very little intercellular substance; these probably are of more recent origin. This type of growth is well marked about certain of the large meningeal vessels and perhaps the small individual whorls surround tiny vessels. Between the denser patches where the cells are not so numerous, other patches of delicate fibrillar network with stellate and fusiform cells are found. Giant cells and mitosis are not observed in the meningeal growth.

Sections through the large hemorrhagic focus show a certain amount of necrosis and a slight infiltration with polymorphonuclear leucocytes and a few fibrin deposits. One large artery of the meninges is found filled by a recently formed thrombus, but otherwise the vessels are negative. The tumor tissue contains only a few small recognizable vessels.

Spinal Medulla: The upper segments of the spinal cord reveal identical findings as described, except that the tumor tissue here is not especially delicate. The loose fibrillar network is not a feature. The arachnoid is slightly thickened. The same affinity of the growth for the vessels and nerves as described above is also noticeable. Otherwise the cord is negative and there is not the slightest invasion of the nervous tissue proper.

Hypophysis: The hypophysis is surrounded by a thin layer of tissue similar to that described in the meninges. Its capsule is thickened. The gland is congested but invasion is absent.

Other sections of the brain, especially those of the right lateral ventricle and the right wall of the third ventricle and the fourth ven-

tricle, are negative. One is unable to trace a direct connection between the main tumor and that of the meninges.

A phosphotungstic acid hematoxylin stain of the meningeal portion of the tumor, shows beautiful glial fibrils especially in the loose and delicate portions of the growth. The coarse intercellular substance takes a yellowish brown color suggestive of collagen. With Mallory's aniline blue stain the entire meningeal tissue is stained red.

The microscopic examination of the other organs revealed nothing of interest in this connection.

Comment

In this case there were symptoms referable to the central nervous system for months. The trouble for which the patient sought glasses was probably an early symptom of the brain tumor. The later developments of projectile vomiting, severe headaches and generalized convulsions terminating in lethargy together with markedly elevated nerve heads, were quite characteristic of brain tumor. The changes in the reflexes were of little value in localizing the growth, and the X-ray of the cranium gave no help.

At necropsy one was not surprised to find what was considered a rather thick exudate in the leptomeninges of the base of the brain and about the spinal cord, especially in view of the repeated lumbar punctures. Material was therefore taken for culture. The large tumor of the left basal ganglia grossly presented nothing remarkable. The hydrocephalus involving the lateral and third ventricles apparently was obstructive in type and certainly due in part to the tumor partially obstructing the orifice of the cerebral aqueduct. The relationship between the large primary tumor and the meningeal changes was not thought of at the postmortem examination.

Microscopically, the bulk of the primary tumor was a typical glioma, but the peculiar small central area, the changes in the ventricular walls and in the meninges, presented very unusual features.

The one confusing feature in the primary growth was the nature of the small area composed of interlacing bundles of large cells. The structure seemed to be apart from the main tumor tissue, by which it was surrounded, and rather sharply demarcated. Numerous sections of the block containing this area revealed no clear transition from the delicate glial tissue and that of the nodule, but in places it was per-

haps suggestive. From the differential stains this nodule was not of connective tissue origin. Because of the site of the nodule and its staining reaction it probably should be considered a part of the glioma and in this respect quite atypical. On the other hand, structurally this area somewhat resembles the tumors of the eighth nerve.

Unfortunately, in the sections it was not possible to trace the extension of the tumor into the meninges. It can safely be concluded, that the tumor did not reach the meninges by way of the cerebral aqueduct and fourth ventricle. Extension along the vessels which penetrate the base of the brain was not found. It is highly probable then, that the growth extended down the left wall of the third ventricle and pierced the thin ventricular floor thus gaining access to the subarachnoid space. Because the nature of the process was not appreciated until the sections were examined, the thin tissue of the floor of the third ventricle was not sectioned.

The coarse character of the growth in large areas in the leptomeninges and in the choroid plexus overlying the tumor, and of the growth composing the large granulations of the ventricular walls, was somewhat similar. In places, especially about the optic chiasm, unmistakable and characteristic gliomatous tissue was found. The transitions of the very delicate into the coarse and more cellular tumor tissue were easily followed in this particular group of sections. Elsewhere the bulk of the growth did not appear like that of a glioma, but in places somewhat resembled an endothelioma of the meninges. But even in these areas on careful inspection and with the aid of special stains, glial fibrils were demonstrated. In this respect the meningeal tumor growth is more or less like many of the reported cases. A characteristic feature in the writer's case was the sharp limitation to the subarachnoid space and cisterna, while invasion in the brain, spinal cord, nerves and vessels was absent.

The cells of the cerebrospinal fluid were counted only on one occasion and the character of the cells, if at all unusual, was not mentioned.

As an afterthought, the slight protrusion of the right eyeball could have been caused by a localized retro-orbital growth from an extension of the glial tissue along the optic nerve into the orbit.

This case then falls definitely into the group of secondary gliomatosis of the leptomeninges and for the most part it is rather typical.

Summary

As pointed out by Firor and Ford and as verified in the above abstracts and case report, the clinical picture of the diffuse involvement of the leptomeninges presented variable features, and was characterized by three main groups of symptoms. The most important symptoms, perhaps, were evidence of brain tumor, signs of cord lesions and paralysis of the cranial nerves. Symptoms of meningeal irritation were occasionally present. A diffuse symptomatology may be present as in the case of deLange and Smid and when so, it is very confusing. Tumor cells were occasionally found in the cerebrospinal fluid and perhaps may aid in the diagnosis, provided one has the condition in mind. The ages of the patients ranged from 11 months to 45 years, but this malady is especially frequent among the young.

In the majority of the cases abstracted by Firor and Ford and by the writer, the site of the primary tumors was in relation to the ventricles, but in several instances the spinal cord was the site of the primary growth. The primary neoplasms histologically were not especially unusual, while the extensions into the meninges and choroid plexuses tended to be more cellular and coarse in structure and unlike the usual appearing gliomas and, to a certain extent, the primary tumors. It seems then that the tumor glial tissue may markedly change its character when in a new environment, such as the leptomeninges, ventricular walls or choroid plexuses. This change and the more cellular meningeal growth appear to have been the chief reason why these cases are regarded by some as sarcomatous. Glial fibrils were considered present in all but Spiller's case (see Firor and Ford's paper). In these instances the site of predilection of the meningeal infiltration was about the base of the brain and cerebellum, and about the spinal medulla. A noteworthy feature was the sharp limitation to the subarachnoid space. The disease appears to be primarily localized in the central nervous system. Invasion and distortion of the spinal medulla or brain has been present in only a few cases, but either or both may be very extensive. The meningeal growth has been confused with a chronic meningitis during the postmortem examination by a large number of pathologists. The meninges of the convexities of the cerebrum were often free of infiltration. The growth was often found following along the nerves

and vessels. In the writer's case the small nodule in the center of the tumor of the basal ganglia stands as an exceptional finding.

The secondary effects of the meningeal tumors were not unlike those due to an organized meningitis. The disturbance caused by the circulation of the cerebrospinal fluid was clearly indicated by the ink injection in Firor and Ford's second case of gliomatosis. Dilated ventricles were commonly found, as well as changes due to chronically increased intracranial pressure.

It seems most likely that the essential mode of dissemination in the leptomeninges of this variety of tumors is by extension rather than by floating tumor cells in the cerebrospinal fluid. Undoubtedly both processes occur as only the latter can explain the distant metastasis under the temporal lobe in Mees' case.

One should be very skeptical of the "diffuse sarcomatosis" or "chronic meningitis" of the pia-arachnoid in the presence of a primary glial tumor of the brain or spinal cord, when the proper differential stains have been omitted, especially phosphotungstic acid hematoxylin. The disease is undoubtedly more frequent than the eighteen reported cases indicate, in view of the large group of "sarcomatosis" of the leptomeninges that has been incompletely studied. Löwenberg in his lengthy discussion of the subject includes certain cases which, when examined in the original, are at best perhaps only remotely related forms.

Conclusion

Diffuse secondary gliomatosis of the cerebrospinal leptomeninges is a distinct pathologic entity.

The writer desires to thank Dr. Jacob Wolfe, Dr. William H. Mayer and Dr. John H. Alexander for the use of the clinical data and also Mr. Carl F. Thilo for his help in translating certain of the references.

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DESCRIPTION OF PLATES

Plates 21–22

Fig. 1. A high magnification of the primary glioma; note the sharply defined mitotic figure and another above and to the right in poor focus.

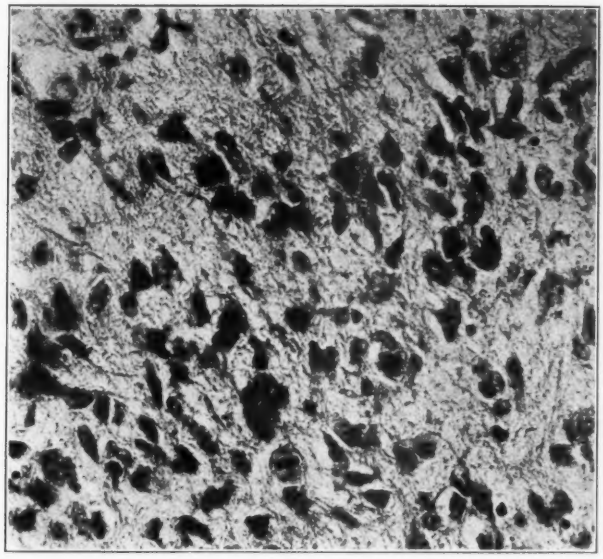
Fig. 2. A high magnification of the glial extension in the leptomeninges near the optic chiasm.

Fig. 3. A low magnification of the subarachnoid infiltration about the brain stem. This shows the slightly thickened arachnoid above and the more delicate tumor tissue in the upper half merging with the more cellular and coarser growth below.

Fig. 4. A low magnification of a very coarse area of the glial tumor growth in the subarachnoid space under one temporal lobe.

Fig. 5. A low magnification of the peculiar circumscribed area found in the primary tumor.

Fig. 6. High magnification from Fig. 5 showing details; note the mitotic figure in the upper left corner and the giant cell on the right. Glial fibrils were not found.



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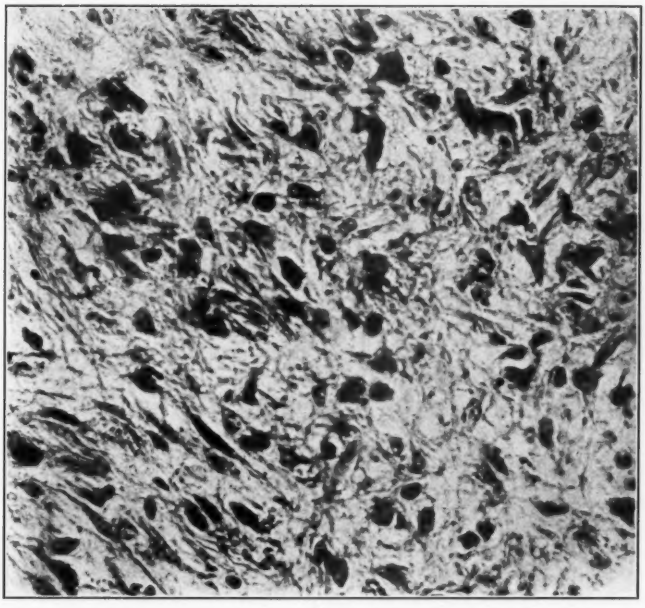

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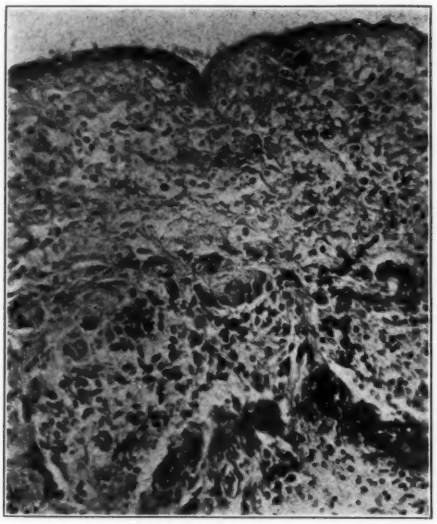

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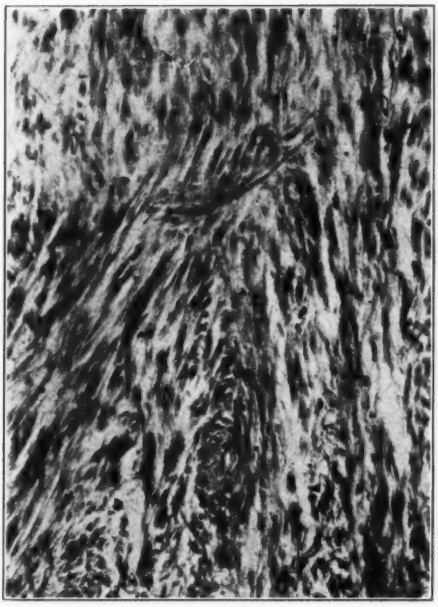

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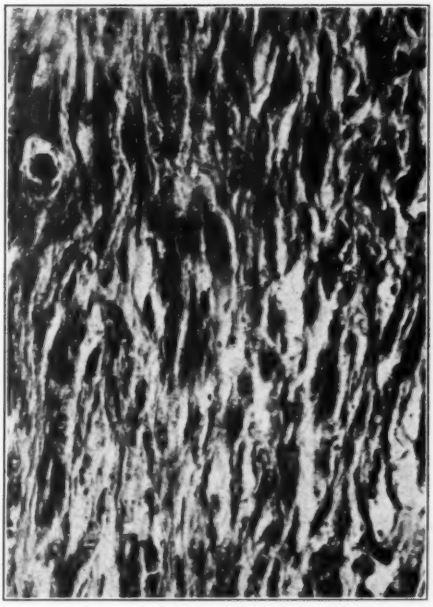

6

Brannan

Secondary Gliomatosis of the Leptomeninges

A RARE CASE OF MULTIPLE CONGENITAL ATRESIA OF THE ILEUM WITH COMPLETE SEPARATION OF A SEGMENT OF BOWEL *

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The subject of congenital atresia and stenosis of the intestinal tract is one upon which no small amount of literature has been written. One of the most comprehensive and critical articles is that of Kuliga [1] in 1903, in which he has collected from the literature previous to that date 185 cases of various types. Since then many more cases have been reported and a great deal of literature has been written concerning the etiology of this somewhat rare condition. The various theories advanced and explanations offered may be divided more or less roughly into two main groups. This division, however, is not absolute as it is altogether possible that in certain cases the final effect may be the result of a combination of causes.

The first group includes those theories in which the cause is supposed to be some malformation or arrest in the course of an otherwise normal development. Briefly they are as follows:

1. Developmental anomalies of the mesenteric vessels. (Wyss suggests that failure of the mesenteric vessels to develop properly is responsible for a corresponding maldevelopment of the bowel with subsequent stenosis or atresia.)
2. Pressure on the duodenum at the point where it passes through the mesocolon (Hess).
3. Forrer suggests that owing to a deficient secretion of bile during intra-uterine life, there is insufficient material to produce proper expansion of the bowel.
4. Hypertrophy of the valvulae conniventes has been mentioned by Hammer and Wyss.
5. Traction of a persistent omphalomesenteric duct by which the loop of bowel, which has been extruded into the root of the

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umbilical cord, is prevented from returning into the abdominal cavity, thus becoming strangled by the closure of the umbilical ring (Ahlfeld [2]).

6. Closely related to 5 is the more recent view of Seisser [3] in which he suggests the additional possibility of an overdistention of the developing cecum, while it is still in the root of the umbilical cord, thus interfering with its retraction into the abdominal cavity. To this he adds the possibility of a diminution in the expansile property of the umbilical ring, probably due to a premature or excessive twisting of the umbilical cord.
7. Another view is that mentioned by Birnbaum,[4] *viz.*, "that the most likely cause of stenosis and atresia of the ileum is that the process of atrophy and involution of the vitelline duct has become excessive and involved the intestine at this point."
8. Persistence of embryologic, physiologic epithelial occlusions of the intestine, particularly of the duodenum, is claimed by Tandler,[5] Kreuter,[6] and Forssner [7] to be the cause.
9. Somewhat closely related to the last theory is the more recent view of Anders [8] that occlusions may result through the complicated process of development of the duodenum and surrounding organs.

To the second group belong those theories in which the cause is said to be the result of some intra-uterine disease. Among others may be mentioned the following:

1. Volvulus (in cases reported by Schottelius and Küttner).
2. Intussusception (attributed by Chiari to be the cause in his case).
3. Fetal enteritis (Markwald).
4. Fetal peritonitis (of which perhaps Theremin [13] is the chief advocate).
5. Other rather uncommon theories belonging to this group include those in which the atresia is attributed to pressure on the bowel from without from some neighboring structure such as an alveolar carcinoma of the liver (Wiederhofer), the hypertrophied head of the pancreas (Hirschsprung), or a cyst in the ileocecal fossa (Schott). A discussion of these various theories will be considered later.

Report of a Case

V., an illegitimate, premature male child, was born Jan. 31, 1924, and on the following day, February 1, was admitted to the Anna Kinderspital, Graz, to which I am indebted for the following clinical notes.

Clinical History. Parents were reported healthy. Two sisters aged 3 years and 9 months, respectively, also were healthy. Labor was spontaneous with duration of two hours. Immediately after the child was born it vomited a blackish material resembling meconium. This vomiting continued with short intermissions for nearly twenty-four hours. No meconium was passed per rectum. The child had taken no nourishment nor had it made any attempt to nurse.

Physical Examination. The child presented the general appearance of a premature, but fairly strong and well-nourished infant. Weight on admission was 2,000 gm. (4 lbs., 6 oz.); temperature 38.8 C (101.4 F); and pulse 140. Examination of the cutaneous, skeletal, nervous, respiratory, circulatory, lymphatic, and genito-urinary systems revealed no significant findings. The umbilical stump was still moist. The abdomen was level with the anterior chest wall. By palpation of the abdomen nothing abnormal could be detected. On attempting to give the child fluid with a spoon it vomited almost immediately. A thin rubber tube was introduced into the rectum for a distance of 20 cm. but neither flatus nor feces were expelled. Upon withdrawal, there adhered to the tube small particles of whitish mucoid material in which were a few fine reddish shreds. The clinical diagnosis was congenital intestinal atresia.

Operation on Feb. 1, 1924 (by Prof. Erlacher). Without an anesthetic, the abdomen was opened by a left rectus incision. Upon opening the peritoneum some clear yellowish fluid escaped. A loop of widely distended bowel presented itself in the operation wound. This proved to be the jejunum which ended distally in a blind sac. From here on the bowel presented a series of alternating dilatations and cord-like atresias. The upper and lower ends of the operation wound were then closed, leaving only a small opening just below the level of the umbilicus. Through this wound the parietal peritoneum was stitched to the skin, and the distal end of the distended loop of jejunum was then sutured to the edges of this opening. A

dressing was applied and the child was left without nourishment for twenty-four hours. The vomiting continued.

On the following day, February 2, by means of the thermocautery a slit-like opening was made in the portion of the jejunum visible in the operation wound. A considerable amount of meconium mixed with bile immediately squirted out of the opening thus made. The general appearance of the child was comparatively good. Temperature normal. Following this operation all vomiting ceased, the child nursed from the breast regularly, and passed by the artificial anus four to five stools a day. These stools resembled those of a normal breast-fed infant, and in stained preparations showed chiefly gram-positive diplococci. The temperature remained around 37.0 C. On the fourth day following the operation (Feb. 6), the child appeared pale, with sunken eyes and refused to nurse. The operation wound showed no evidence of healing. The abdomen was soft, and the stool unchanged. In the morning of the following day (Feb. 7) the child died.

Postmortem Examination

The necropsy was performed by Dr. H. Glatz.

The body is that of a male child 39 cm. long. Aural cartilages are still soft. Finger nails do not reach to the finger tips. The whole body is still thickly covered with lanugo. Anterior fontanelle is 2 x 2 cm. Below the umbilicus and slightly to the left of the midline is a recent longitudinal operation wound about 3 cm. in length. Inserted in the center of this wound is a portion of intestine in the wall of which is an opening leading into the bowel lumen. The edges of this wound are reddish black and sloughing. Examination of the brain reveals nothing worthy of note. Upon opening the thorax, both lungs are seen to be voluminous, firm and do not collapse. Pleura is dark bluish red, dull and covered with a thin fibrinous exudate with fibrinous adhesions between the various lobes. The cut surface of all lobes is dark red and nodular and shows comparatively little air-holding tissue. The pericardial cavity contains an increased amount of fluid with thick fibrinous deposits on the pericardium. The heart and large vessels show no malformations. The mouth, pharynx, larynx, trachea and esophagus appear normal. Upon opening the peritoneal cavity the abdominal viscera are seen to be covered with a hemorrhagic, fibrinous exudate by means of

which the coils of intestine are more or less adherent to one another. Nothing worthy of note is found in the liver, pancreas or spleen. The suprarenal glands are large; the cortex contains little lipoid material and the medulla is soft and hemorrhagic. The kidneys show quite definite fetal lobulations.

A sound can readily be passed from the stomach through the pylorus, duodenum and jejunum and out through the artificial anus in the abdominal wall. This portion of the intestine is widely distended, averaging about 2.5 cm. in diameter, and contains considerable yellowish soupy material. The wall varies from 1 to 3 mm. in thickness and is quite firm.

Two cm. below the artificial anus the lumen of the intestine ends in a blind sac, while the bowel itself continues in a thin cord-like obliterated strand not more than 1 mm. in diameter, which after a distance of 1 cm. again expands into an oval, patent segment 10 x 6 mm. This expansion narrows rather abruptly again into another cord-like obliteration. From this point to the ileocecal valve the ileum consists of a series of five alternating expansions and obliterations. The expanded segments vary from 4 to 10 mm. in diameter, and from 1.5 to 4 cm. in length. In all but the last two obliterated portions, a definite cord-like structure is visible connecting the patent segments. In these last two portions, *viz.*, immediately proximal and distal to the last patent segment of ileum, there is not the slightest trace of intestinal tract to be found, the expanded segment being sausage-shaped with smooth rounded ends and supplied with only a narrow band of mesentery. From the ileocecal valve to the anus the intestine is again patent, the colon averaging about 4 to 5 mm. in diameter. The distance from the duodeno-jejunal junction to the first atresia is 40 cm. The mesenteric attachment for the jejunum, ileum and colon as far as the splenic flexure extends from the region of the lower border of the terminal portion of the duodenum downward and toward the right, a little to the right of the midline. The mesentery itself measures 1.5 to 3 cm. in length in the areas supplying the patent segments of bowel, but the portions corresponding to the obliterated strands are very much shorter, in some cases not more than 2 mm. in length. Extending from the summit of the loop of sigmoid colon, is a thin semitransparent band of tissue which passes upward and toward the right and crosses over the small intestine in the region of the third atresia in the mesentery of which

it disappears (Fig. 1). No other fibrous or peritoneal bands can be seen. The mesenteric glands are somewhat large, grayish red and soft.

After a very careful removal of the gastro-intestinal tract from cardia to anus, together with the mesentery, there remained attached to the posterior abdominal wall just below the right kidney, a small portion of bowel with rounded ends, 18 x 9 x 4 mm. in size. This presented the same macroscopic appearance as the segment previously described just proximal to the ileocecal valve. All the patent portions of small intestine below the first atresia are tightly filled with a semisolid, grayish white, finely granular material. Both testes are in the scrotum. Bladder, ureters and urethra show nothing worthy of note.

Anatomic Diagnoses. Laparotomy wound with fecal fistula leading into lower jejunum just below the umbilicus. Multiple atresia of the ileum with dilatation and hypertrophy of the jejunum. Isolation of a segment of bowel from the mesentery. General fibrinous, hemorrhagic peritonitis. Bilateral bronchopneumonia with fibrinous pleurisy and pericarditis. Bilateral suprarenal medullary hemorrhage. Prematurity (approximately eight months).

Microscopic Examination

All the abdominal and thoracic viscera were fixed in 10 per cent formalin, and later portions were chosen for microscopic examination. With the exception of the examination of the intestinal tract (including the isolated segment of bowel) nothing bearing on the etiology of the atresias was found. The portion of the bowel examined microscopically included the isolated segment and the region of the first two atresias 4.5 cm. in length (Fig. 2). The findings in the case of the former will be described first.

This segment was embedded in paraffin and cut in longitudinal serial sections which were stained with hematoxylin and eosin and with van Gieson's connective tissue stain. The latter stain gave the more information (Fig. 3). The wall of the segment consists essentially of a muscular coat, submucosa and mucosa. In places there is seen in addition a definite serosa and subserous connective tissue. At other points both these elements are lacking. On the posterior surface, namely, that of attachment to the posterior abdominal wall,

no serosa is visible, but there is an abundance of extramuscular vascular connective tissue. The muscular coat consists of two distinct layers separated throughout by a very thin layer of connective tissue. On the whole, these layers present the usual thickness seen in the small intestine of newborn infants, but in certain regions, particularly at both ends of the segment, they are considerably thinner and yet quite definite. In the middle portion of the segment the fibers of the inner layer run in a definite circular direction, while those of the outer layer are longitudinally arranged. Toward the end of the segment, the inner fibers are cut in a more longitudinal plane while those of the outer layer are cut more transversely. The submucosa forms a definite layer throughout, at times of medium thickness, at other times considerably thicker. The degree of vascularity of the submucosa also varies considerably, and in places there is rather marked lymphocytic infiltration. In the mucosa one can trace the muscularis mucosae in almost every section. In places this is quite thick, in other places it is reduced to a few scattered fibers, while in still other areas it has disappeared entirely. Lining the segment is a rather irregular mucosa containing tubules of which the cells are chiefly cylindrical with basal nuclei and in some areas are distended with mucus. In certain places the tubules are considerably flattened out, while in others the epithelium has disappeared entirely. Here it may be noted that the epithelium shows a greater loss of continuity than does the muscularis mucosae. The latter is always present where the former is found and always extends some little distance beyond the point at which the epithelium ceases. The lumen of the segment is filled with a granular amorphous material which stains a pale red in hematoxylin and eosin preparations, and in which are found fragments of cell nuclei.

The portion of bowel immediately below the fecal fistula chosen for microscopic examination, is shown in Fig. 2. The first portion of the proximal enlargement which represents the blind end of the distended jejunum was cut transversely (Fig. 2*a*). The second portion (Fig. 2*b*) was cut in serial longitudinal sections. Serial transverse sections were also cut from the section (Fig. 2*c*). Single sections were taken from *d* and also from the region of the sigmoid colon. The transverse sections from segment *a* show the bowel here to have a normal structure, all the various coats being represented and in normal proportions. At this point there is no evidence of

muscular hypertrophy which is quite noticeable in the more widely distended proximal portion. There are, however, some acute inflammatory changes in the serous surface and subserous tissues, which can be explained by the general peritonitis which was present, and also by the close proximity of this segment of bowel to the operation wound.

The serial longitudinal sections from *b* show that the blind end of this portion of bowel is lined with a definite epithelium, muscularis mucosae and submucous connective tissue, none of which at any point shows any break in continuity. The muscle fibers, however, which proximally form two distinct and normal layers, become more and more irregular in their arrangement as the atresia is reached, until they unite from all sides to form an almost solid bundle of irregular muscle tissue; in such a bundle are found a few blood vessels and scattered connective tissue fibers, the whole being surrounded by a thin layer of subserous connective tissue and, except for the area of mesenteric attachment, by serosa. After a distance of about 0.5 mm. the muscle fibers take on a more regular arrangement, forming definite outer longitudinal and inner circular layers. Between the two layers the nerve plexuses of Auerbach are seen to be quite prominent. Here appears a definite central vascular connective tissue core which extends throughout the whole of the first obliterated segment.

The enlargement at *c* (Fig. 2) has a definite lumen and all the layers in the wall of the bowel are here represented. The transition from this patent segment to the second atresia is seen to take place in a similar manner to that described above, except that in this case there is no break in the continuity of the submucosa which is continued into the obliterated strand as a core inside the two layers of the muscular coat.

In the rather widely distended segment at *d* (Fig. 2) there is nothing particular to note except that the epithelium tends to be more flattened out as though under pressure and has in places disappeared entirely, while the muscularis mucosae has remained intact throughout. Here also there is to be seen a moderate degree of lymphocytic infiltration in the submucosa. Likewise the amorphous granular material contained in the lumen of this and other patent portions resembles that seen in the isolated segment first described.

Microscopic examination of the colon reveals nothing worthy of

note except that the epithelial folds are rather prominent, the lumen is comparatively small and contains only a small amount of débris.

In the mesentery supplying both the obliterated and patent portions of bowel, are seen a goodly number of blood vessels which show no pathologic change. There is here, however, a certain amount of edema, polymorphonuclear leucocytic infiltration and extravasation of blood which may be interpreted as part of the acute general peritonitis. The mesenteric glands also show a moderate amount of edema and interstitial hemorrhage.

In chloroform extracts of the bowel contents below the first atresia, no bile pigment could be demonstrated. Definitely positive results, however, were obtained from the material contained in the distended proximal portion.

Discussion

From the above detailed report, the points which perhaps merit the greatest emphasis include: the presence of an isolated segment of intestine below the right kidney; the multiplicity of atresias in the remainder of the bowel, in two of which the separation is complete; the extreme shortness of the mesentery in the region of some of the atresias; the presence of a fibrous band which passes over the third segment of obliterated bowel; and finally the absence of bile pigment below the first atresia. The first of these findings, namely, that of the isolated segment is, so far as we have been able to discover, peculiar to this case. Several cases of multiple intestinal atresia have been reported and also quite a number of cases in which the intestinal tract is completely separated at some point or other. Likewise cases of multiple atresia are reported in which the mesentery in the region of the atresia is abnormally short, but in no report were we able to discover a description of an isolated segment such as was found here. The two cases which perhaps most closely resemble this are quoted by Kuliga[1]; one was reported by Marrignes and quoted by Meckel (1812) and the other was reported by Schottelius (1881). In both of these cases, the intestinal defects were multiple and the separation was complete, but no reference is made to any isolated segment such as we have described. Unfortunately, at the time of writing, the original articles were not available.

When we come to a consideration of the etiology of this condition,

we find that the majority of the theories mentioned above do not offer any satisfactory explanation of the peculiar findings in this particular case. Let us consider first the theories included under group 1, *viz.*, those which have as a basis some developmental malformation.

1. The first of these theories, namely, that of Wyss, that the failure of the intestine to develop at certain points is due to an absence of mesenteric vessels in the corresponding segments of mesentery can be excluded here, as not only do the mesenteric vessels in these areas appear quite normal microscopically, but there is also a good vascular supply in the connective tissue core of the obliterated portions of intestine (Figs. 4 and 5).

2. Likewise the suggestion of Hess that the pressure of the mesocolon on the duodenum may lead to stenosis may here be excluded, because in this case the atresias were all below that level, the first atresia being 40 cm. beyond the duodeno-jejunal junction.

3. So also the theory of Forrer that the atresia results from a deficient secretion of bile seems to require little consideration, as it is purely hypothetical. If, as Forrer supposes, the expansion of the developing bowel is dependent upon the supply of bile, it would be difficult to understand why some of the segments in our case between the atresias should be so well distended while other areas have neither contents nor epithelial lining. It is true, however, that in these patent segments no bile pigment could be demonstrated, but in our opinion this is due to the fact that the atresias had occurred before the secretion of bile had been established. This according to Keibel and Mall [9] would probably be before the fourth month of intra-uterine life.

4. Concerning the possibility of hypertrophy of the valvulae conniventes resulting in stenosis or atresia, we would say that while this possibility may have existed in the cases reported by Hammer and Wyss it is altogether unlikely to have played any part in our case, first because of the situation of the atresias, *viz.*, in the ileum which normally is not supplied with these structures, and second because the atresias here consist of a series of solid strands and not of diaphragmatic membranes, as is sometimes the case.

5 and 6. Theories 5 and 6 may be disposed of briefly together as both have to do with the failure of the intestinal loop to return to the abdominal cavity during intra-uterine life. Since this develop-

mental defect did not exist in our case, it cannot be assigned the cause of the atresias described.

7. Likewise the explanation mentioned by Birnbaum [4] requires little consideration here, as one cannot conceive of an excessive involution of the vitelline duct involving the intestine at more than one point, which would be necessary to explain the multiple atresias in our case.

8. The theory that intestinal atresias may arise through failure of embryologic, physiologic epithelial occclusions to disappear was first advanced by Tandler [5] who stated, however, that he did not wish this view to be considered as more than a hypothesis. This hypothesis, nevertheless, was based on observations made by Tandler during the examination of a series of eleven embryos ranging in length from 7 to 21.6 mm. These were all studied in serial sections and Tandler observed that in three embryos 8.5, 11, and 12.5 mm. long respectively, the proliferation of the duodenal epithelium in places was so great as to bring about an obliteration of the original lumen. In older embryos this epithelial occlusion had disappeared and the duodenum again possessed a lumen. Similar epithelial occlusions were also observed in the esophagus, and confirmed by Kreuter.[6] In 1907 Forssner [7] published a rather extensive work on this subject in which he not only confirms Tandler's findings in relation to the duodenum, but attempts to explain the process by which such occlusions may lead to stenosis or atresia. His explanation is as follows: If the epithelial occlusion has not disappeared by the time folds begin to appear in the duodenal wall as the result of proliferation of the mesenchymal cells into the epithelium, it may thus happen that the mesenchyme may grow completely through the mass of epithelial cells before the lumen has reappeared. In this way an atresia is produced in the bowel which consists of connective tissue embedded in epithelium. If the connective tissue bridge is thin, the result is only a membranous obstruction; if it is thicker, a cord-like atresia results. If the connective tissue bridge is incomplete or if at some point it is so weak that it ruptures, an incomplete membrane is the outcome. Forssner then continues, "Through alterations of these primary types during fetal life all other forms of stenosis and atresia may develop." He attempts on this basis to explain not only the occlusions in the duodenum, but also those in the remainder of the intestinal tract where no embryonic occlusions have been demon-

strated, assuming that such occlusions may occasionally develop and thus form the foundation for a permanent atresia or stenosis. This theory has been rather strongly assailed by Schridde [10] who was unable to demonstrate any such embryonic epithelial occlusions, and therefore concludes "that the view that congenital atresias of the bowel are traceable to such occlusions is untenable in every respect."

By a careful examination of Schridde's articles,[11] however, it is quite evident that at the time of his investigations his attention was focused on the examination of the embryonic esophagus concerning which he gives rather minute data, whereas a detailed description of his findings concerning the duodenum or remainder of the intestinal tract is entirely lacking. This being the case, it is a question whether he has sufficient basis for his conclusion that epithelial occlusions in the intestinal tract do not exist, and therefore cannot give rise to atresia or stenosis.

Even if we take it for granted that such epithelial occlusions do occur normally in the embryonic duodenum as claimed by Tandler and Forssner, it is altogether unlikely that the atresias in the case here reported could be traceable to such occlusions for two reasons: first, because of their position which is in the ileum, a location in which no epithelial occlusions have been demonstrated; and second, because of their length, whole stretches of bowel being obliterated while the occlusions reported by these writers extended over a comparatively very short distance. We therefore feel justified in assuming that some other factor was responsible for the lesions reported and that the theory just discussed does not apply in this particular case.

9. Likewise the theory of Anders [8] can here be similarly excluded, as it applies only to atresias in the region of the duodenum. This theory may be explained briefly as follows: The development of the duodenum is a very complicated process, involving the combination of the rotation of this portion of the intestinal tract with the development in its wall of important structures such as the common bile duct, the pancreatic duct and the ampulla of Vater, while in the immediate neighborhood are developing the pancreas, liver and pylorus. According to this author, if now from any cause there should arise a disturbance in the finely graded correlation in the growth of these various parts (if, for example, the regio papillaris

should lag behind, or the rotation of the duodenum proceed too rapidly) the whole general arrangement of these structures may be altered and an atresia or stenosis may result. To what extent this theory is based on actual observations made by Anders we are not prepared to say. In the article from which the above notes are extracted, the author does not give any details of his investigations, nor unfortunately does he give any reference to the publication of such details.

Thus from a review of the theories which attempt to explain congenital intestinal atresias from the standpoint of developmental defects, we are unable to find any one theory which would seem to offer a satisfactory explanation of the findings in our particular case. We, therefore, pass on to a consideration of the theories belonging to the second group, namely, those in which the atresias are supposed to be the result of some intra-uterine disease.

1. Volvulus, we believe, can be excluded first of all because there was nothing suggesting a volvulus to be found and second because of the multiplicity of the atresias. On the other hand it might be argued that a volvulus, once present, need not necessarily persist throughout the period of intra-uterine life and that its absence at birth does not exclude the possibility of its former existence. Such a possibility, in our opinion, seems to be quite improbable. It might also be mentioned here that in discussing this question, Kuliga [1] gives it as his opinion that in the majority of cases of intestinal atresia in which a volvulus was found, the volvulus was the result and not the cause of the atresia. In this connection he quotes Henning's experiment (but gives no reference) in which he was able to produce a volvulus in a cadaver by distending the bowel with water above a ligature.

2. The possibility of an intussusception being the cause in our case is so remote as to require no discussion here. The same may be said of the other rare theories in which the atresias have been attributed to a tumor of the liver, the hypertrophied head of the pancreas or a cyst in the ileocecal fossa.

3. Fetal enteritis on the other hand requires more consideration. In a rather lengthy discussion of this subject Kuliga [1] assumes that in order that an inflammation of the intestinal tract result in atresia there must first be a destruction of the mucosa with subsequent obliteration of the lumen by proliferation of the submucous connec-

tive tissue. As possible etiologic factors he mentions mechanical and chemical irritation, pyogenic bacteria and the more chronic infections of tuberculosis and syphilis, with the further possibility in certain cases of a tendency to submucous hemorrhage with stripping up of the mucosa which subsequently disappears leaving the way open for the connective tissue proliferation mentioned above. All of these etiologic factors, however, he considers to be quite improbable. Whether this question can be disposed of as readily as this writer would assume is problematical. While we have no evidence to show that fetal enteritis did at any time exist in our case, at the same time we do not believe that its possibility can absolutely be excluded. While some might interpret the lymphocytic infiltration in the wall of the patent segment of bowel noted above, as an evidence of a former enteritis we are of the opinion that this is rather the expression of irritation, by the intestinal content which being unable to alter its position in the bowel, would act as a mild irritant over a long period of time. The fact, also, that one segment of bowel was completely severed from the remainder and that in the two atresias immediately proximal to the ileocecal valve, no remains of intestinal wall whatever could be found, would lead one to believe that the causative factor in the production of such separations was some force acting from without, rather than an inflammatory process inside the bowel. This brings us to a consideration of the one remaining theory mentioned above, namely, (4) that of fetal peritonitis.

That fetal peritonitis does occur has long been recognized.[12] To it Theremin [13] ascribed the cause of intestinal atresia as long ago as 1877. Ballantyne [14] draws attention to this condition as an etiologic factor in the production of certain pelvic and abdominal malformations such as congenital flexions of the uterus and congenital obliteration of the bile ducts. In this connection we recall the case of a premature infant dying in the Hospital for Sick Children, Toronto, thirty-six hours after birth in which at necropsy we found a definite pelvic peritonitis from which we recovered an unidentified, anerobic, gas-producing, gram-positive bacillus. That the peritonitis was of intra-uterine origin was determined by the fact that microscopically the peritonitic adhesions already showed fibroblastic proliferation. That such adhesions, if occurring early in intra-uterine life, could

produce malformation of the pelvic viscera is quite conceivable, and if of the pelvic viscera why not of the intestinal tract?

In the case here reported there was found a band or adhesion extending from the summit of the sigmoid colon to the mesentery of the ileum, crossing the ileum in the region of the third atresia (Fig. 1). The most likely explanation of the presence of this band is that it is of inflammatory origin and that it was present at a very early stage of intra-uterine life while the intestinal tract was still very small. It is quite conceivable that pressure of such a band on an intestinal tract with only a potential lumen could result in atrophy of the intestinal mucosa at that point with subsequent fusion of the submucous connective tissue. This view is further strengthened by the fact that the mesentery at this point is very short, suggesting that the pressure of the band hindered its development. It is true that only one such band was present while there were several atresias. In order to explain the remaining atresias on the same basis it is necessary to assume that several other such bands had been present, all of which had disappeared after the establishment of the atresias, probably as a result of fusion with the peritoneum. In a similar manner the isolated segment of gut could gradually have been pinched off from the remainder of the intestinal tract and become adherent to the peritoneum just below the right kidney. In the absence, therefore, of any other satisfactory explanation as to the cause of these findings we are forced to the conclusion that the atresias in this particular case, at least, are the result of an intra-uterine peritonitis.

Summary

An interesting case of multiple atresia of the ileum with complete separation of a segment of bowel is here reported. Such an isolated segment is, so far as we have been able to determine, peculiar to this case. The absence of bile pigment below the upper atresia would indicate that the occlusions had occurred before the third or fourth month of intra-uterine life. The presence of a peritoneal adhesion suggests that the cause of these atresias was an early intra-uterine peritonitis.

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DESCRIPTION OF PLATES

Plates 23–25

Fig. 1. Small gut showing dilated jejunum (with operation wound) and multiple atresias of ileum.

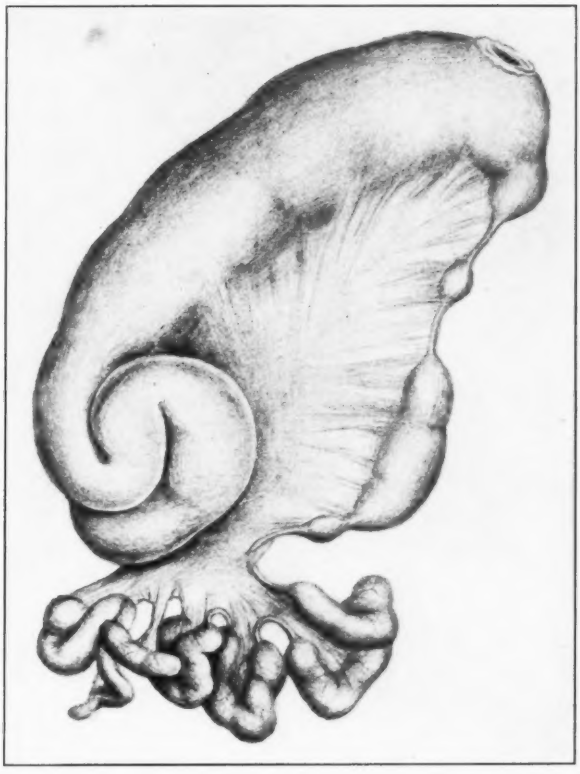
Fig. 2. Sketch of first two atresias showing portions chosen for microscopic study (see text).

Fig. 3. Microscopic section taken longitudinally through the isolated segment of gut (van Gieson's stain). (Insert shows natural size of the segment.)

a. serous coat.
b. outer longitudinal muscle.
c. inner circular muscle.
d. submucous connective tissue.
e. muscularis mucosae.
f. epithelium.

Fig. 4. Microscopic section taken longitudinally through the blind end of the jejunum (see Fig. 2 *b*).
Note the presence of all coats in the patent portion and the vascular connective tissue core in the obliterated segment (van Gieson's stain).

Fig. 5. Microscopic transverse section through the first atresia. Note the outer longitudinal and inner circular muscle fibers with the vascular connective tissue core. The outer ragged covering is largely inflammatory, due to the recent acute peritonitis.



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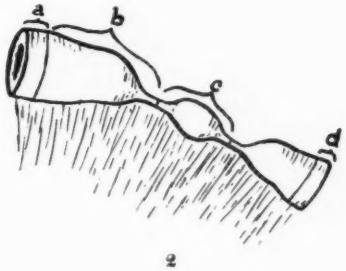

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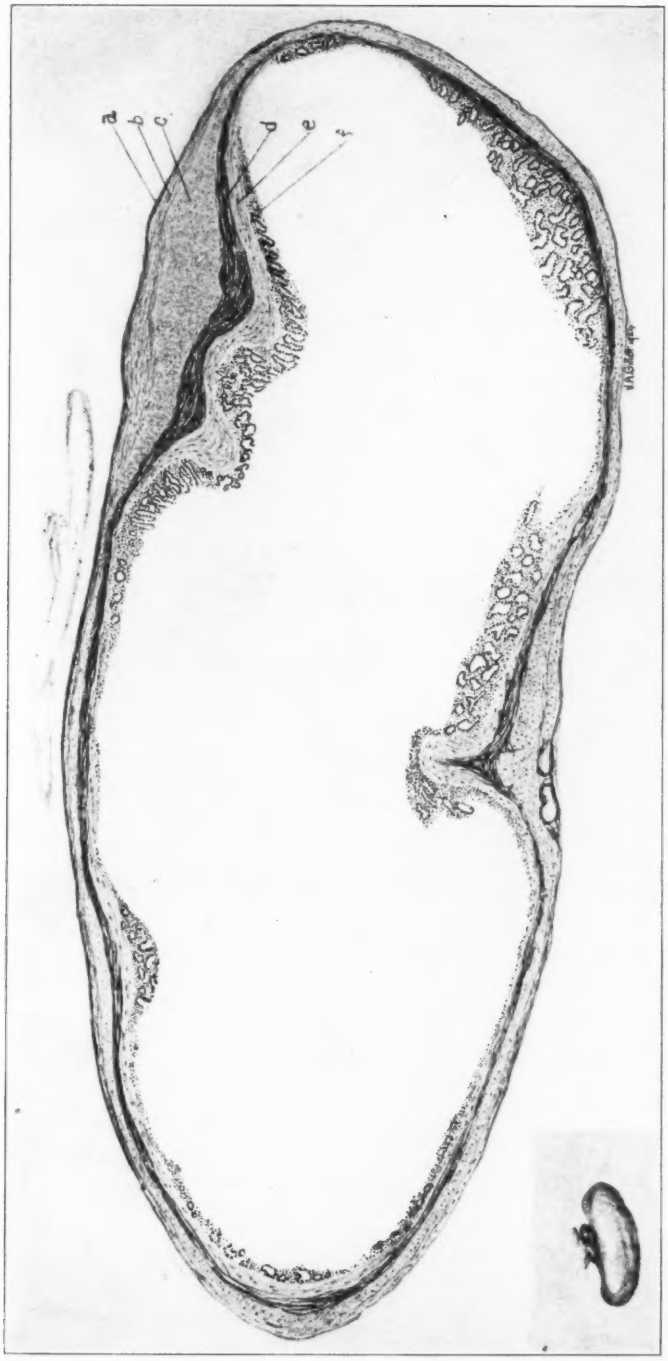

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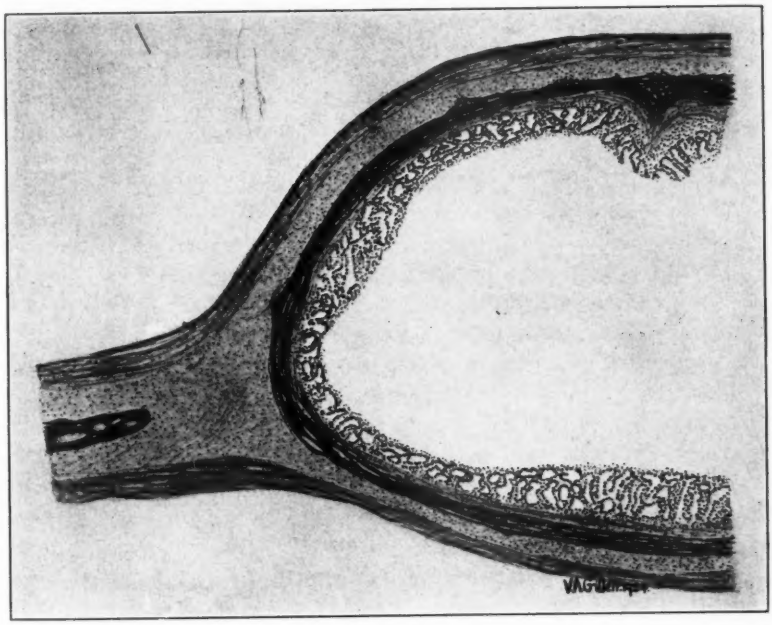

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5

ADENOMA OF THE SALIVARY GLAND *

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Of the tumors of the salivary gland perhaps adenomas number among the most rare. In fact their occurrence was doubted by Volkman. In 1892, however, Nasse [1] in his work on "Tumors of the Salivary Gland" described a tumor which could definitely be classed as an adenoma. Previous to that time, many cases reported as adenomas of the salivary gland were, according to our present classification, obviously mixed tumors which, among other structures, contained areas of adenomatous character.

That adenomas of the salivary gland occur can no longer be doubted, for there are several cases (less than ten) on record which are sufficiently accurate in their description and obvious in their histology to establish definitely their identity as such. Because of the complexity of mixed tumors, one is not justified in classing any given tumor of the parotid gland as adenomatous without first subjecting the entire growth to a careful examination for any of the characteristics of a mixed tumor; for even though the bulk of the tumor is adenomatous, a minute area in which there is evidence of any characteristics of mixed tumors, such as cartilage, immediately designates that tumor as of the mixed variety. It may be possible, perhaps, that many of the mixed tumors are at first adenomatous; in fact one may ascribe the rarity of adenomas to the possibility that some of them become mixed before they are presented for histologic examination.

According to Lambret,[2] there are two types of adenoma of the salivary gland: intraglandular, in which the neoplastic tissue develops primarily at the expense of epithelial elements of the gland, and extraglandular, in which the adenomatous tissue arises in the ramifications of Steno's duct within the gland.

In the so-called extraglandular type, the canaliculi are dilated and stand out grossly or microscopically as cystic areas wholly or partially filled with epithelial proliferations. The papillae are made

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up of stalks of connective tissue growing from the wall of the duct and are covered with one to several layers of epithelial cells. Each stalk branches in a more or less arboreal fashion so that, as far as structure is concerned, it is similar to the well known papilliferous cystadenoma of the ovary. In certain areas the epithelium which is usually cuboidal becomes stratified and forms small projections which extend into the cystic cavity. These areas are found both along the papillae and the epithelial lining of the duct between the papillomatous projections. There is no tendency for the neoplastic tissue to break through the duct wall and invade the surrounding parenchyma. As can be expected, there are no acini in this extraglandular type.

In the intraglandular type we do not find the cyst formation nor does the adenomatous tissue assume a papillomatous architecture. Where the extraglandular type reminds one of the papilliferous cystadenoma of the ovary in structure, the intraglandular type is structurally reminiscent of the circumscribed adenoma of the thyroid gland. We find a highly cellular area containing acini both formed and formative, the whole surrounded by a definite connective tissue capsule.

It is this intraglandular type that is presented by our case of which a detailed description is here given.

Clinical History

The patient, a negro girl 18 years of age, was admitted to this hospital complaining of "a growth in front of the right ear." Three years previously, she noticed a small lump just anterior to the right ear. This growth gradually increased in size until it had reached its present dimensions (about that of a walnut). During the past year she complained for the first time of pain in this area, which was never severe and was always localized to the immediate area of the tumor. Except for three miscarriages, her history is otherwise negative.

Physical examination reveals a tumor about the size of a walnut in the right parotid region. It has definite borders, is firm in consistence and has a slightly irregular surface. It is not attached to the skin and is only slightly adherent at its upper pole to the underlying gland. It is not tender to palpation. There are no other glandular enlargements. The remainder of the physical examination is substantially negative.

Operation. With a diagnosis of "fibroma of the parotid," the patient was operated upon the following day. A small perpendicular incision was made directly over the tumor which was found just beneath the skin, only slightly embedded within the gland and surrounded by a well formed connective tissue capsule. It was easily enucleated, being found only loosely adherent at its upper pole to the underlying gland. The gland itself was apparently entirely normal.

Gross Examination

The specimen consists of a tumor mass about the size of a walnut with a small amount of glandular tissue attached to its upper pole. The tumor is well encapsulated and has a smooth, or slightly irregular surface. It is pale pink in color and has a firm elastic consistence. On section, it cuts easily and reveals a homogeneous, compact, cellular-appearing surface, brownish gray in color, with narrow bands of connective tissue running here and there throughout the tumor as though dividing it into lobules. There is an area of slight injection in that portion of the capsule which lies immediately adjacent to the neoplastic tissue. The entire tumor consists of one type of tissue.

Histologic Examination

Sections were stained with hematoxylin-eosin and mucus stains. The neoplastic tissue is composed of dark blue-staining cells, closely packed and ranging from spindle-shaped to round or oval. So marked are these features that one is inclined at first glance to think of a tumor of connective tissue origin (Fig. 1). Further examination reveals differentiation as described below.

The growth is surrounded by a definite capsule of rather broad dimensions with the only notable feature of having apparently two portions. The more peripheral is composed of old, dense connective tissue in which are contained a few blood and lymph vessels; the portion, much narrower but quite definite, immediately adjacent to the tumor is of much more recent development, and has a looser structure due to the distended lymph spaces and the greater number of blood channels.

Extending into the tumor are many prolongations of the more adjacent portions of the capsule, which form well defined septa dividing the tumor into irregular but definite lobules. Here and there near the periphery of the tumor are sundry isolated areas of the same kind of tissue as that making up the tumor. These areas are completely surrounded by young connective tissue and give the appearance of having been "snipped" off by the connective tissue fibers rather than of direct invasion (Fig. 2).

Each lobule besides being separated from the other lobules by the septa mentioned above, is surrounded by a delicate band of connec-

tive tissue more or less loosely approximated to the tumor cells. There is no evidence of any nutritional or nerve elements except for an occasional small blood channel.

The shape and arrangement of the tumor cells vary markedly. Some are definitely spindle-shaped with dark staining nuclei; others are polygonal; still others are rounded or oval with dark round nuclei and a proportionate amount of slightly granular cytoplasm (Fig. 2).

The definitely spindle-shaped cells tend to run in parallel lines but as they take on a polygonal and oval shape there is a tendency for them to be arranged in whorls. Many of these whorls are composed of solid masses of cells arranged in a circular fashion, but some contain spaces in their centers, forming thereby structures strongly suggestive of young acini. This impression is substantiated by the fact that in each lobule there are numerous examples of definite acini composed of cuboidal, typically glandular cells surrounded by a membrana propria and containing, as shown by appropriate staining, mucus in their lumina.

All through the tissue this tendency to the formation of acini is finally completed in the formation of the definite acinus described. The section is especially interesting from that standpoint. One sees, in a measure, a portion of the genesis of an acinus; first the spindle-shaped cells arranged in parallel rows, these gradually forming whorls as the cells take on oval or round shape; the formation of small lumina surrounded by closely packed cells with those nearest the lumen taking on an undoubted cuboidal, glandular appearance; and finally the enlarging lumen filled with mucus, the more definitely glandular cells, the membrana propria and the formed acinus.

The diagnosis is, therefore, adenoma.

Summary

An adenoma of the parotid gland encountered in an 18-year old girl is reported. The duration was three years with slow growth and slight pain. It was encapsulated and microscopic examination showed the epithelial elements varying in their arrangement from undifferentiated columns of cells to formed acini.

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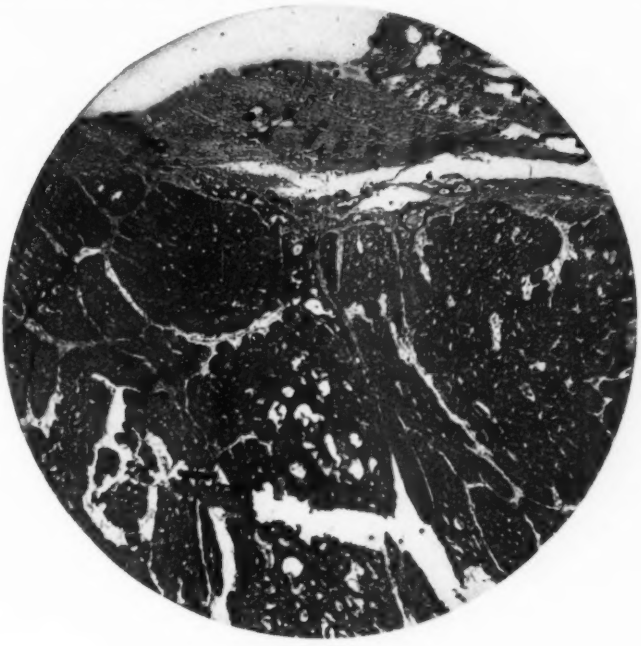
DESCRIPTION OF PLATE

Plate 26

Fig. 1. Showing general topography of tumor. X 36.

Fig. 2. Higher magnification showing clearly the various shaped cells making up the tumor and the formation of definite acini, many of which contain mucus in their lumina. X 166.



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2

Schutz Adenoma of the Salivary Gland

CYSTITIS EMPHYSEMATOSA *

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The occurrence of gas-containing cysts in the wall of the urinary bladder is exceedingly rare, if we exclude those gas cyst formations which are caused by putrefactive postmortem processes or which are a part of an infection with gas gangrene bacilli. This alteration of the bladder, known as cystitis emphysematosa, is described eleven times in the recent literature of human pathology (Eisenlohr,[1] Camargo,[2] Kedrowsky,[3] Ruppanner,[4] Schöneberg [5] with three cases, Nowicki [6] [7] [8] with four cases) and is mentioned as a disease of animals only by Kitt [9] who found it accidentally in a few cows and by Olt [10] who observed it once in a sow's bladder. The emphysematoses of the intestinal and vaginal mucosa, to which cystitis emphysematosa is related etiologically and histologically, are by far not so rare. Pneumatosis cystoides is a rather frequent finding in the intestine and mesentery of pigs. Also colpitis emphysematosa, usually found in the human vagina, is much more common than cystitis emphysematosa (Nowicki,[8] Gröndahl,[11] Näslund [12]).

The pathologic alterations in the bladder wall in cystitis emphysematosa are, according to the descriptions of the above-mentioned authors, as follows. Macroscopically, single cysts or small groups of cysts of pinhead to pea-size are present on the inner surface of the bladder (Nowicki, Ruppanner, Kedrowsky, Camargo) and less often the whole surface is more or less covered by those cysts (Eisenlohr, Nowicki). Signs of an inflammatory reaction of the mucosa and submucosa are very inconstant. Sometimes the bladder has the appearance of an acute inflammation (hyperemic vessels, hemorrhages, epithelial defects, and mucous, turbid and bloody urine); there is only some discoloration due to old blood pigment as a sign of a chronic inflammatory process; or the mucosa looks normal. Concerning the involvement of the different layers of the bladder wall, cross-sections show marked variations. In some cases only the

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mucosa is affected; in others also the submucosa contains cysts; while in a third group, cysts are present in the whole wall of the bladder producing a spongy appearance.

Microscopically, the cysts either are lined by endothelial cells and multinuclear giant cells derived from the endothelium, or they have no cellular lining but are surrounded by several layers of homogeneous fibers. The first-mentioned type of cysts is interpreted as distended lymphatics and the other type as enlarged tissue spaces because of the lack of a cellular lining. Cysts of both kinds are generally found. Concerning the inflammatory reactions observed, only two cases (Nowicki, Kedrowsky) showed acute inflammatory symptoms. In the rest more or less frequent nests of lymphocytes were observed in the interstitial tissue revealing the chronic character of the process. The epithelial lining of the bladder was defective in some cases, being mostly intact but showing atrophic or hypertrophic changes. All authors except Ruppanner succeeded in demonstrating the presence of bacteria in the tissue. Usually these were gram-negative rods, but cocci were also found. According to the conclusions of Nowicki, the disease may start as an acute inflammation with a great tendency toward being chronic and advancing into the deeper layers of the bladder wall. He rejects the conception of Schöneberg who interprets the cystitis emphysematosa as a purely agonal alteration of the bladder, on account of the large number of giant cells and the lymphocytic infiltrations observed.

The etiology of this disease as well as the corresponding emphysematoses of the intestinal and vaginal mucosa is still under discussion. There are two main theories. The first one supported only by a few authors explains the cystic formations as the results of a mechanical process in which gas, swallowed with the food or produced in the lumen of these organs by bacteria, is pressed through small lesions of the epithelium into the tissue. But artificial injections of air into the intestinal wall fails to produce a lasting formation of gas cysts. The lack of an experimental substantiation of this mechanical theory and the frequent finding of bacteria in the tissue of emphysematous mucous membranes by later investigators have given support to the microbiologic theory. In cultures of the tissue, many authors (Jäger,[13] Nowicki, Näslund, etc.) were able to cultivate gas-producing organisms, mostly gram-negative rods which showed the characteristic qualities of *Bacillus coli*. Jäger and Näslund produced

emphysematous alterations by injections of those cultures into the intestinal wall. Nowicki [8] therefore contends in his last paper that cystitis emphysematosa is caused by an infection of the bladder wall with *Bacillus coli* which may invade this organ either by way of the blood stream or through lesions in the epithelium. As predisposing factors, are mentioned passive hyperemia in the pelvis caused by the pressure of tumors or a pregnant uterus on the pelvic vessels, general circulatory disturbances (Nowicki,[8] Aschoff,[15] Kaufmann,[14] Delafield and Prudden [16]) or hyperglycemia (Rossi [18]). It is well known that the increased sugar content of the blood, as found in diabetes and after feeding a food rich in sugar (Schöneberg, Rossi), produces in the tissue a favorable medium for bacteria because the sugar diffuses under these pathologic conditions from the blood into the lymph. The importance of this factor was shown by Rossi who succeeded in producing pneumatosis cystoides in a high percentage (37.3 per cent) of pigs after feeding them whey which contains much lactose (4.3 per cent). Olt, however, thought that the presence of the bacteria in the tissue might not always be necessary, but that possibly their enzymes absorbed through a damaged mucosa might sometimes be sufficient to cause these lesions.

A noteworthy feature of this disease is, furthermore, its apparent restriction to the female sex. Nowicki who mentioned this fact did not try to make any comment, although there is evidently no reason for this limitation either to a specific anatomic structure as in colpitis emphysematosa or to any of the suspected etiologic factors.

I received some time ago the bladder of a female dog from the Physiological Department (Loyola Medical School) for examination. This bladder, showing the alterations of a cystitis emphysematosa as never observed before, seemed to offer me the favorable opportunity for a reëxamination of the histologic findings and the etiologic suggestions of the previous investigators.

The dog being under observation for several weeks and apparently absolutely normal was killed for experimental purposes. The examination of the abdominal organs showed a rudimentary pancreas occurring as a retroperitoneal node the size of a hazelnut. To obtain some urine for a sugar test, the bladder was opened and the following unusual condition was found.

Macroscopic Examination: The lumen of the bladder is very much diminished by the presence of a layer about 1 cm. thick of grayish

white, gas-filled cysts ranging in size from that of a pinhead to a pea. These cover the whole inside of the bladder and leave deep crevices and fissures between the cysts. The consistence is like an air cushion or a sponge and a marked crepitation is felt on pressure. In a cross-section of the bladder wall the mucosa and submucosa have a honeycomb appearance. The muscularis is macroscopically normal (Figs. 1 and 2).

Microscopic Examination: The considerably folded mucosa shows a marked hyperplasia of the epithelium. Very frequently the epithelium has formed solid nests and cysts lined by several layers of epithelial cells and filled with gas underneath the covering epithelium. Surrounding the cell nests a few lymphocytic infiltrations are present. The thickness of the submucosa is much increased by the presence of these cysts which do not contain any demonstrable material. They are irregular and vary in size and shape. Their walls are generally lined by a single layer of flat endothelial cells, and only in a few cysts the lining is partly formed by giant cells. The cysts are embedded in a delicate framework of loose connective tissue. Here and there even this is lacking and the cysts are separated only by a septum of two layers of endothelial cells. Lymphocytic infiltrations are small and rarely found in the framework of connective tissue (Figs. 3 and 4). The microscopic examination of the muscularis reveals the presence of many elongated cysts in the spaces of this tissue. These cysts, however, show mostly a lining of several concentric layers of homogeneous fibers. Sometimes the cysts are filled with erythrocytes, probably due to the rupture of small capillaries. Bacteria cannot be detected in the sections stained by Gram's method and with polychrome methylene blue in spite of a very thorough examination.

In the kidney, glycogen infiltration of the tubular epithelial cells is present. The liver shows an extremely fatty degeneration. The pancreas rudiment is composed of glands with an acinar structure without any Langerhans' islands. An increase of the blood sugar above 200 mgm. was found in the blood. The urine gave a strongly positive test for sugar. Bacterial cultures were unfortunately not made.

The *pathologic diagnoses* were diabetes mellitus; hypoplasia of the pancreas; fatty degeneration of the liver; and cystitis emphysema-

tosa. The histologic findings of this case did not differ from those described by previous investigators. The cysts were apparently mostly distended lymphatics in the mucosa and submucosa and enlarged tissue spaces in the muscularis. A different origin must be suspected for the cysts in the mucosa which were lined by epithelial cells. Probably they were derived from inversions and folds of the mucosa as already suggested for similar cysts of the vaginal mucosa in vaginitis emphysematosa.

The most important feature of this case is the lack of bacteria in the tissue in spite of the remarkable extension of the emphysematous process and the marked hyperglycemia which would produce a favorable medium in the tissue for growth and gas formation especially for *Bacillus coli* (Hitschmann and Lindenthal,[19] Wells[17]). This fact stands strongly against the above-mentioned theory of Nowicki. It is impossible to explain the cystitis emphysematosa in the case just described as being due to a bacterial origin in the tissue. There remain two more explanations as to the origin of the gas in the bladder wall. First, enzymes produced by bacteria in the urine absorbed into the bladder wall may here split off CO_2 from the carbohydrates present (Olt); and second, an explanation which is purely theoretical, a disturbance of the sugar metabolism in the tissue of the bladder wall may be presupposed to have caused the production of gas. Olt's suggestion is the more plausible one of the two.

The restriction of this rare disease to the female sex cannot be explained satisfactorily. But it has to be considered that only a small number of cases were observed. The limitation, therefore, may be accidental. Moreover, predisposing factors are more frequent in the female sex than in the male, such as circulatory disturbances in the pelvic organs by tumors and disturbances of the sugar metabolism as encountered in goiter and pregnancy.

Conclusions

1. The report of a case with cystitis emphysematosa in a female dog is given.

2. Bacteria could not be detected in the tissue, therefore a direct infectious origin could not be substantiated.

3. The production of gas may be due to absorption of bacterial enzymes from the urine containing glucose.

4. The restriction of the cystitis emphysematosa to the female sex is possibly only accidental due to the small number of the cases observed and the greater frequency of the predisposing factors in the female sex.

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DESCRIPTION OF PLATES

PLATES 27–28

FIG. 1. Inner surface of the bladder. X 3.

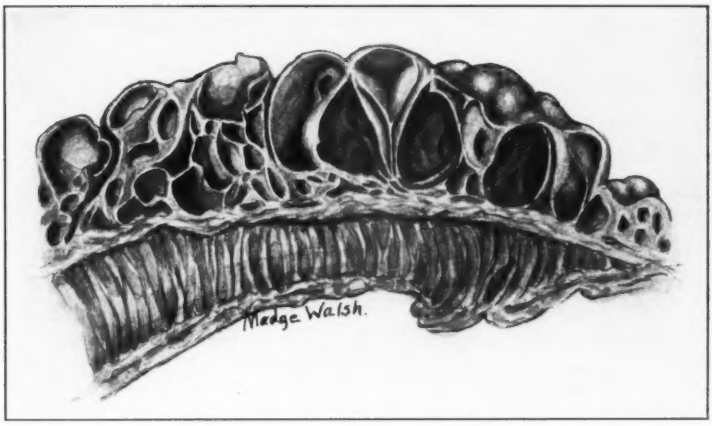
FIG. 2. Cross-section through the bladder wall. X 3.

FIG. 3. Bladder wall showing gas cysts in the mucosa, submucosa and muscularis. Low magnification.

FIG. 4. Gas cysts in the mucosa and submucosa. High magnification.

1

Hueper Cystitis Emphysematosa



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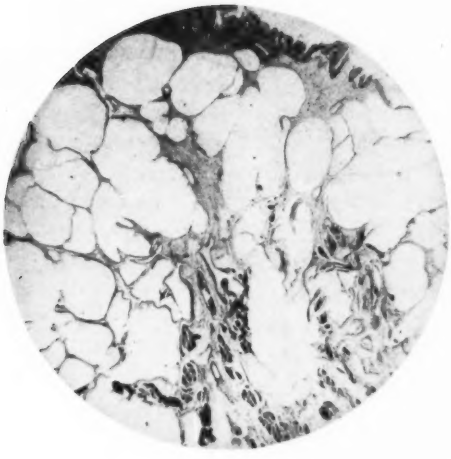

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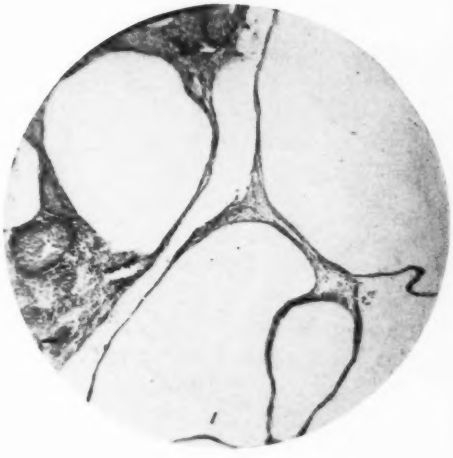

4

STUDIES ON THE EFFECT OF CHLOROFORM ON THE LIVER OF RABBITS *

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INTRODUCTION

After chloroform was introduced as an anesthetic in general practice, instances were soon reported of death following general anesthesia within three to five days and accompanied by very significant symptoms. According to the necropsy records of these cases, fatty changes of the parenchymatous organs, especially of the liver, were found. These unexpected deaths were explained by the delayed poisoning effect of the chloroform. Experimental investigations were soon started in this matter.

LITERATURE

Whipple and his collaborators [1, 2, 3, 4, 5, 6, 7, 8, 9] tried to find the connection between the poisoning power of the chloroform and the effect of fasting, various diets, drugs and chemical agents in addition to the morphologic changes due to chloroform. Wells [10, 11, 12] studied the question especially from a pathochemical point of view. Graham [13, 14, 15] emphasized the rôle of hydrochloric acid produced by decomposition of chloroform as a factor effecting the development of necrosis. Opie and his coworkers [16, 17, 18, 19, 20] carried out experiments with regard to morphologic changes as well as the consequences of different diets. He succeeded in producing a beginning liver cirrhosis by simultaneous injections of chloroform and bacteriotoxins. T. Ogata,[21] S. Ogata,[22] and lately Jaffé,[23] investigated the effect of chloroform especially with regard to the development of cirrhotic changes in the liver. Muskens [24] issued an important publication on the morphologic development of necrosis.

METHODS

Our own experiments, carried out on rabbits, are divided into the acute and the chronic poisonings. Ophüls [25] calls attention to the

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fact that the cirrhotic changes in the liver of rabbits should be accepted with suspicion on account of the frequency of coccidiosis of rabbits. But Gye and Purdy [26] point out, on the other hand, that the lesions due to coccidiosis are circumscribed and easily distinguished from the diffuse cirrhotic changes such as are caused by chloroform. Rabbits are nevertheless suitable for such experiments as their liver is especially sensitive to different poisons and cirrhotic changes are comparatively easily produced.

Pure "chloroform for anesthesia" was injected subcutaneously. Though some experimentalists prefer prolonged and repeated anesthesia, the use of injections has the advantage of exact doses quickly and conveniently given.

ACUTE CHLOROFORM POISONING

For experiments in acute poisoning twelve rabbits were used, each weighing between 1400 and 1800 gm. Chloroform was injected subcutaneously as follows: eight of them received 1.25 cc., one 1.0 cc., one 0.5 cc. and two were twice given 0.5 cc. Eight of the twelve rabbits were killed after various periods by a blow on the back of the neck and four were found dead. In cases of acute poisoning, the duration of life varied between three hours and four and one-half days after the injection.

Necropsy was done in every case as soon as possible. Pieces of liver, kidney and heart were fixed in Zenker's solution, 4 per cent formaldehyde and in 95 per cent alcohol. Those fixed in alcohol were embedded in celloidin, the rest in paraffin. For staining the slides the following methods were used: eosin-methylene blue, hematoxylin-eosin, hematoxylin-van Gieson, Mallory's phosphotungstic acid hematoxylin, Mallory's aniline blue connective tissue stain, Foot's method for impregnation, Weigert's stain for elastic fibers, Mallory's iron reaction, ammonium sulphide reaction and v. Kossa's silver nitrate reaction. For testing different fats, scarlet red and hematoxylin, Nile blue sulphate and Fischler's stain for fatty acids and soaps were used. Glycogen was tested by Best's carmine stain on sections fixed in alcohol and embedded in celloidin.

RESULTS OF ACUTE POISONING

With regard to the considerable difference existing between the course of acute and chronic poisoning and the changes of the liver

due to the same, it seems reasonable to discuss the two kinds of poisoning separately. The changes caused by acute poisoning are sufficiently uniform to be discussed together.

In rabbits killed three and six hours after injection, no changes in the liver are found with the naked eye. In all the other cases, the liver is swollen and friable, with smooth surface on which characteristic markings are seen. There are spots corresponding in size to the lobules of the liver; the center of each is grayish, circumscribed by a small yellowish ring which in turn is surrounded by an area having the color of normal liver tissue. On section, the markings are similar to those seen on the external surface. The heart and kidneys are grayish and friable. The lungs are congested. No necrosis in the pancreas or in the mesenteric fat tissue is present.

No important histologic changes are found in the liver of the rabbits killed three and six hours after injection, except that the cells are lighter colored and more vacuolated than normal. These slight changes are more pronounced in the center of the lobule. Glycogen is found in many of the liver cells, a larger amount being in those around the central veins. There is no evidence of fat either in the liver cells or in endothelial cells.

In those rabbits killed and examined twenty-four hours and later after poisoning, well marked injury of the liver is present in every case. Reports of these are given and grouped according to the different staining methods as follows.

Marked changes are noticed in the liver of rabbits, killed twenty-four hours following the injection, in preparations stained with eosin-methylene blue and hematoxylin-eosin. Well marked necrosis is present in the central two-thirds of each lobule. The liver cells of this region are somewhat smaller than normal with sharp outline and homogeneous cytoplasm deeply stained with eosin. The necrotic liver cells are mostly arranged in columns, but in some places this arrangement is hardly noticeable. The nuclei of such necrotic cells show different changes. The first stage of these lesions is the disappearance of the nucleolus, with the simultaneous appearance of fragments of the chromatin substance on the inner surface of the nucleus membrane. More advanced necrotic processes are found in most cells, *i.e.*, a shrunken, pyknotic, deeply stained clump of chromatin is in the place of the former nucleus. The outlines of the sinusoids among the necrotic liver cells cannot be recog-

nized definitely. The endothelial cells of the central veins and of the sinusoids do not give any evidence of necrotic changes. The endothelial cells of the sinusoids are swollen, oval, sometimes round and lie freely in the spaces between the necrotic liver cells and most of these spaces are filled with red blood corpuscles. The number of endothelial cells does not seem to be increased and mitotic figures are not present. Very striking is the relatively small number of polymorphonuclear leucocytes between the necrotic cells. The peripheral liver cells, the bile ducts and the perilobular connective tissue do not show any changes. The border between the necrotic and normal liver cells is rather definite. Here, there are to be seen one or two layers of swollen, lightly stained, vacuolated liver cells. The nuclei of these cells are fairly well stained.

In the liver of the rabbit found dead two days after poisoning, all these changes are more pronounced. Almost the whole lobule is necrotic and only two or three layers of normal liver cells are left on the periphery of the lobule. The border between the necrotic and normal cells is well marked, but vacuolated cells, described above, are seldom present. The necrosis is more advanced than in the case of the rabbits killed after twenty-four hours. The necrotic cells are no longer arranged in columns. These cells are similar to drops of protoplasm in that they are small, without any definite shape and red in color; small, pyknotic nuclei are rare. In most of such necrotic cells there is only a dim shadow of the nucleus or even this is missing. The endothelial cells of the central veins do not suffer any changes, but the endothelial cells of the sinusoids are swollen, in most places desquamated and lie free between the necrotic cells. The number of endothelial cells seems to be increased, but mitotic processes could not be proved. The endothelial cells are in some places quite near the necrotic cells and partially surround them. The necrotic liver cells are in other places surrounded by four to five endothelial cells, giving the impression of the development of foreign body giant cells. Polymorphonuclear leucocytes are infrequently found, and in some places ten to twelve lymphocytes are aggregated.

The findings are similar in the liver of the two rabbits which died spontaneously two and one-half days after poisoning, with the difference that in the necrotic areas typical giant cells are present, which seem to have incorporated the remains of necrotic liver cells. The

endothelial cells in the necrotic areas are increased in number, but also those between the normal liver cells seem to be swollen. Pyknotic nuclei or clumps of chromatin are not common in the necrotic cells which are homogeneously stained with eosin and contain few vacuoles. The number of polymorphonuclear leucocytes is relatively small, but some of them have invaded the necrotic liver cells. In the necrotic as well as normal liver cells, small brownish granules, probably of bile pigment, are present.

In the liver of the three rabbits killed three days after poisoning, the necrosis is located in the central one-half and three-fourths of the lobule. Chromatin is hardly recognizable in the necrotic cells. In two of these cases the necrotic cells have granules stained bluish with hematoxylin. In place of some necrotic liver cells there are round, blue masses surrounded by typical foreign body giant cells. The endothelial cells in the necrotic areas are certainly increased in number, some showing mitotic figures. The endothelial cells are remarkably swollen between the normal liver cells. Contrary to what was stated in the cases above which were examined sooner after poisoning, there are more polymorphonuclear leucocytes and mast cells in the necrotic places. The swollen, vacuolated cells, described above, differ in number on the border between necrotic and normal areas in the three cases investigated. In the normal liver cells many mitotic processes are taking place even at the periphery. Mitotic figures often seem to be irregular. Brown pigment granules are in the necrotic and normal liver cells. Lymphocytes and endothelial leucocytes are more numerous in the perilobular connective tissue than normally. Mitosis is found in the interstices in one of these cases.

The findings in the rabbit which died three and one-half days after poisoning are on the whole similar to the above cases. However, the findings in the rabbit killed four and one-half days after injection, present considerable differences. Very few necrotic cells are in the central one-third of the lobule; endothelial cells are swollen and in increased numbers; and there are a few giant cells. This part of the lobule is constructed mostly of connective tissue fibers and endothelial cells, and for this reason gives the impression of a lightly colored reticulum after the liver cells have fallen out of it. The sinusoids are large and filled with red blood corpuscles. Leucocytes are few in number. Swollen vacuolated cells are at the border

between the necrotic and normal areas. Mitotic figures are found in the normal liver cells.

In order to get pictures suitable for studying the connective tissue, the slides were stained with Mallory's aniline blue, hematoxylin-van Gieson and phosphotungstic acid hematoxylin stains. No important changes are seen in the connective tissue except in the four and one-half days case in which the connective tissue fibers are especially prominent in the central parts. In the impregnation of the fibers, which was tried by the method of Foot, there is no difference in any of the cases. The equal impregnation of small round granules in the necrotic and normal cells is remarkable. These granules seem to be artificial products at first sight, but after thorough investigation they prove to be uniform and located always inside the cells.

Glycogen was tested for with Best's carmine stain in every case, but the test was positive only in those of three and six hours in which necrotic changes were not yet evident. In most liver cells glycogen is present especially in those located in the central parts. Glycogen is not present in the necrotic or in the normal cells of a liver with necrosis.

In frozen sections stained with scarlet red, the following findings are present: At the end of three and six hours after poisoning neither in the liver cells nor in the endothelial cells is there a considerable amount of fat to be found. There are finer and more irregular droplets in the necrotic cells in the liver of cases one day and older. It is remarkable that the fat staining is absent in the necrotic cells lying in the immediate neighborhood of the central veins. We can find large fat droplets in the swollen and vacuolated cells above mentioned, on the border between the necrotic and normal areas, so that these surround the necrotic places like a deeply stained circle. This layer can be exceptionally wide, so that in every peripheral liver cell fat is present. Fat drops are also to be found in two cases (three and three and one-half days old) in the endothelial cells of the periportal vessels and in the perilobular connective tissue.

Different results are obtained with Nile blue sulphate which was used to determine the characters of the different fats. In the three and six hour cases no fat cells are visible. In two of the other rabbits we could not find any fat staining in the necrotic cells. In the necrotic liver cells in five rabbits the fat is violet in color and in three

deep blue. In the swollen cells surrounding the necrotic area, the fat is stained in two cases vivid red, in five violet and in three others deep blue.

With Fischler's method which serves to point out the fatty acids and soaps, in two of the ten necrotic cases no staining could be obtained. In two livers the result is doubtful, but in six others pronounced black granules and masses are present in the necrotic cells.

With v. Kossa's silver nitrate method which demonstrates the presence of calcium phosphate, in eight of the ten necrotic livers lime salts are demonstrated. The lime salts mostly form fine granules in the necrotic cells, although in two cases there are large round masses which are surrounded by typical foreign body giant cells. We tried to demonstrate iron with a solution of potassium ferricyanide and hydrochloric acid and also with ammonium sulphide. Iron so tested is not visible in the necrotic and normal liver cells or in the endothelial cells.

In all the rabbits of this group parenchymatous degeneration is found only in the heart and kidneys.

DISCUSSION OF ACUTE POISONING AND CONCLUSIONS

Our experiments prove that central liver necrosis can be produced in rabbits without exception by subcutaneous injection of 0.50 to 1.25 cc. of chloroform. The necrosis is hyaline in type and the nuclei are pyknotic. The polymorphonuclear leucocytes are few in number. The endothelial cells do not show necrotic changes; they are swollen, increased in number, surround the necrotic liver cells and form typical foreign body giant cells.

This description corresponds with most of the results obtained by other experimenters and also with the findings in human pathology. We were able to produce the necrotic changes described, although authors of the past, namely, Nothnagel,[27] Ostertag,[28] Strassmann,[29] Ungar and Junkers (cited by Howland and Richards)[40] and lately Teschendorf[30] emphasize the production of only fatty changes in the heart, kidneys and liver in different kinds of animals by prolonged or repeated chloroform narcosis. Doubtless the cause of this is insufficient administration of chloroform. Thus Jaffé[23] could produce only fatty degeneration in the liver with smaller

doses of chloroform, and necrotic changes were present only in limited areas. The observation of Mosiman and Whipple [31] that the pigeon, frog and terrapin are resistant to chloroform, should be mentioned. Different kinds of animals and even individuals are susceptible to chloroform in different degrees. Yet, given the proper amount of chloroform to rabbits, we succeeded in producing in most cases necrosis of the liver cells.

Whipple and Sperry [1] and somewhat later Muskens [24] investigated the development and progress of chloroform necrosis. The first two authors cut out a piece from the liver of a dog anesthetised with chloroform for three hours for the purpose of histologic examination, but no pathologic changes were found. They did the same excision after twenty-four hours and killed the dog after forty-eight hours. The finding in the liver after twenty-four hours was necrosis in the central one-half of the lobule and after forty-eight hours necrosis in the central two-thirds. Muskens [24] did not find any parenchymatous injury either immediately after one hour of anesthesia or three hours later, except a considerable amount of fat granules in the endothelial cells of the sinusoids. Fat in traces was present twelve hours after the anesthesia in the central as well as in the peripheral liver cells. After twenty-four hours the central necrosis was well marked. Our observations agree in all respects with those of these authors. We did not find any central necrosis in the liver of rabbits killed three and six hours after injection, the first evidence of necrosis appearing after twenty-four hours. The only variation from normal in the cases of three and six hours is that the liver cells are faintly stained and more vacuolated, which is especially marked in the central parts. This slight difference is not sufficient to be called a pathologic change. Jaffé [23] speaks of this process as a physiologic one.

Mitosis appears on the third day not only in the liver cells at the border of the necrotic areas but also in the most peripheral. The mitotic figures very often seem to be multiple, a phenomenon which reminds one of the cells of malignant tumors. Four and one-half days after poisoning, the necrotic cells have mostly vanished. Whipple and Sperry [1] observed mitotic figures at the end of the second day, necrotic cells on the sixth day and complete regeneration on the eleventh day. Carraro [32] injected chloroform into the portal vein of rabbits and thereby caused focal instead of central

necrosis. He observed the beginning of the regeneration early in the third day. Jaffé [23] pointed out that most of the central necrosis areas regenerate completely and only a very small proportion of them is replaced by granulation tissue.

It is a noteworthy fact that glycogen can be stained by Best's carmine stain only in the three and six hour cases. But there is no proof of glycogen either in the necrotic or in the normal liver cells in cases where central necrosis had already appeared. The rabbits were kept on a diet rich in carbohydrates during all the experiments. In addition, 12 to 25 gm. of glucose were given daily through a stomach-tube to three of the rabbits five days before the injection and all the time following it. The necrosis of the liver which developed in these rabbits corresponded fully with that observed in the others. Opie and Alford [19] emphasized the fact that the liver of carbohydrate-fed animals is less sensitive to chloroform and the regeneration is more prompt. Davis and Whipple [6] came to the same conclusions. According to Graham's [14] statement, the liver of puppies is not susceptible to chloroform on account of its rich glycogen storage. But central necrosis can still be produced in these animals after depriving the liver of glycogen by means of starvation or previous injection of phloridzine. Muskens [24] suggests the administration of carbohydrates for therapeutic purposes in human cases for the same reason. The small number of our experiments in which we could not interfere with the development of liver necrosis through carbohydrate feeding, cannot possibly contest with the large series of experiments carried out by the authors mentioned. The total absence of glycogen in the necrotic livers, as observed in our experiments, seems to confirm their statements of the relation between the development of necrosis and the carbohydrate metabolism.

After the examination of fats, we came to the same conclusions as most of the other authors, that a comparatively small amount of fat is present in the necrotic cells. On the contrary, a considerable amount of fat is in the vacuolated cells located at the border between the normal and necrotic areas. Muskens [24] found minute fat granules in the endothelial cells immediately after one hour of anesthesia and also three and twelve hours after the same period of anesthesia. He suggests that this storage is due to the lipemia caused by chloroform anesthesia. Our three and six hour cases do

not seem to prove this. Only one of the three day cases seems to give evidence of fine fat granules in the normal endothelial cells lying between normal liver cells. But in this rabbit as well as in another (three days) fat is also present in the periportal connective tissue and in the endothelial cells. This presence of fat can be explained as being deposited from the central necrotic and the peripheral fatty degenerated liver cells, much more than as the result of lipemia due to chloroform; the presence of advanced necrosis and fatty degeneration seems to confirm the above supposition.

We have already referred to the fact that the fat of the central necrotic and the peripheral degenerated liver cells varies in color when stained with Nile blue sulphate. With the help of Kawamura's [33] findings, we were able to detect the presence of glycerol esters, cholesterol esters and real lipoids in the liver of rabbits poisoned by chloroform. But we could not throw any light on the problem as to why the fats vary in the poisoned liver of different individuals.

Fischler [34, 35, 36] demonstrated the frequent appearance of central necrosis in his Eck-fistula dogs anesthetized by chloroform. He recognized the presence of fatty acids and soaps in the necrotic areas by means of his own method. He emphasizes the important rôle of pancreatic juice in the development of the central liver necrosis based upon these findings. Chloroform injures the liver cells, weakening their vitality so that the fermentative effect of the pancreatic juice exerts itself and consequently the autolysis of the liver cells occurs. On the basis of finding fatty acids and soaps, he concludes that in these places the pancreatic lipase was acting; so he suggests that trypsin also has an important part in the destruction of the liver cells. In further experiments, he immunized Eck-fistula dogs against trypsin and he could not find any evidence of central liver necrosis, even in cases of pancreatic fat necrosis artificially produced. With Fischler's method, not only the fatty acids, but also the lime salts are colored with hematoxylin which is the basis of the stain. Findings with this method are only of value if the efforts to discover lime salts bring a negative result.

According to Hildebrandt,[37] there is no connection between the fermentative action of the pancreatic juice and the development of central liver necrosis. To point out the calcium soaps in our cases, we used Benda's reaction with a saturated copper acetate solution

in water (on material fixed in formalin), but the gross as well as the microscopic reaction done on frozen tissue remained negative.

We applied the silver nitrate reaction described by v. Kossa on material fixed in alcohol and embedded in celloidin, to demonstrate calcium phosphate. We obtained striking results with this method. In eight cases out of the ten in which liver necrosis was present, we found black granules in the necrotic liver cells. One of the two negative rabbits was poisoned for two and the other for four and one-half days. In six cases the lime salt formed very minute granules inside of the necrotic cells, but in two instances large round black masses were visible surrounded by typical foreign body giant cells. Only in the two latter cases were we able to discover lime salts with hematoxylin-eosin and hematoxylin-van Gieson staining; in the other six they were demonstrable only with the silver nitrate reaction. The reason for this is that the lime in the necrotic cells is in very fine granules and so remained unstained by hematoxylin; or else the stained lime granules were mistaken for clumps of chromatin. According to the investigations of Klotz,[38, 39] lime is only visible in slides stained with hematoxylin if it is present in a sufficient quantity. Contrary to this, v. Kossa's reaction is very sensitive and is able to detect even a trace of lime.

Howland and Richards [40] could demonstrate lime salts in only two dogs in central liver necrosis due to chloroform poisoning. Pancreatic necrosis was not present in either case. Dark clumps stained with hematoxylin were visible in the necrotic liver cells, which gave a positive silver nitrate reaction and, therefore, proved to be calcium phosphate.

Whipple and Sperry[1] found pancreatic necrosis in two dogs, but with hematoxylin staining no lime could be discovered in the liver. Fischer's [41] observation stands entirely separate and is hard to explain. He injected 1.2 cc. chloroform into a mouse within eleven days. He found in the liver various degenerative changes and in the periportal connective tissue large and small lime clumps surrounded by giant cells. In the liver cells themselves no lime salts were visible.

We did not find pancreatic fat necrosis in any of our cases and on account of the positive result of v. Kossa's silver nitrate reaction, we consider the black granules and masses stained according to Fischler's instructions to be calcium phosphate instead of soaps. The negative Benda reaction seems to confirm this suggestion as well

as the negative silver nitrate reaction obtained on slides previously treated with hydrochloric acid and acetic acid.

The calcium deposits in our necrotic liver cells are similar to the calcium precipitation in the necrotic cells of the kidney in mercury poisoning. Such precipitation appears in the necrotic liver cells as rapidly as in the kidney in mercury poisoning. The fact is well known that calcium precipitation easily occurs in the body in tissues of weakened vitality.

It is still questionable why investigators do not prove the presence of lime salts in more human and experimental cases. This fact might be explained either by failure to use v. Kossa's reaction which is much more reliable for testing lime than the hematoxylin stain; or the calcium deposit might occur only in rabbits because of the high calcium content of their blood, which is stated by v. Kossa [42] as 1.06 to 2.01 per cent.

v. Kossa made experiments to study the calcification of kidney cells due to different poisonings. He administered iodoform to rabbits through a stomach-tube for a couple of days, but could not find any lime in the kidney cells; on the other hand, there was a considerable amount of calcium in the liver. The liver showed well marked fatty changes and there were fine granules which could not be detected by the hematoxylin stain, but which gave a positive reaction with silver nitrate. To some other rabbits, he gave at the same time iodoform and (subcutaneously) calcium chloride, in consequence of which the calcification occurred sooner and more distinctly. Calcium chloride injections did not seem to affect the liver cells without administration of iodoform. We discovered changes by means of chloroform injections very similar to those found by v. Kossa and as chloroform is in close chemical relation to iodoform, we might say that v. Kossa's findings support our own.

We proved the presence of lime salts in 80 per cent of the rabbits with necrotic liver cells, and hence we draw the conclusion that this must be a characteristic feature of the central liver necrosis due to chloroform.

Bile pigment was present in most of the normal as well as the necrotic liver cells, and it might have caused the appearance of black granules in the liver cells after impregnation.

Iron was visible neither in the necrotic and normal liver cells nor in the endothelial cells. These examinations were necessary to take

into account Brown's [43] findings, according to which the hemosiderin content of the rabbit's liver is increased within twenty-four to forty-eight hours due to autolysis *in vitro*. The pigment is supposedly formed from hemoglobin by the oxidative action of ferments. We could not find any evidence of this in our cases.

CHRONIC CHLOROFORM POISONING

LITERATURE

Many experimentalists have tried to cause chronic chloroform poisoning for investigation of experimental liver cirrhosis.

Thus Mertens (cited by Opie [17]) daily injected subcutaneously 0.1 to 0.2 cc. of chloroform dissolved in paraffin oil. Some of his animals could be kept alive for six months. The liver was firm and granular after this treatment. Microscopically, various necrobiotic cells were found, together with an increased amount of connective tissue around the central veins and between the lobules, which fused at a later stage of the process.

Herter and Williams [44] anesthetized the same dog twenty-four times within eight weeks for one to one and one-half hours each time. Connective tissue was increased in the liver. Joannowics (cited by Jaffé [23]) had the same findings in chronic chloroform poisoning.

Opie [17] administered subcutaneously considerable amounts of chloroform for a long period and found increased connective tissue around the central veins. He maintained that the connection between that and the previous central necrosis is self-evident. He succeeded in producing a more marked increase of connective tissue and also the proliferation of bile ducts by means of simultaneous injections of chloroform and the toxin of *Bacillus coli* or of the streptococcus. According to Opie, the bacteriotoxins promote the development of cirrhotic changes. He could obtain in this way advanced cirrhosis within twenty-three days.

T. Ogata [21] repeated Opie's experiments with negative results.

Jaffé [23] caused various cirrhotic changes by careful, repeated, subcutaneous injections of very small doses of chloroform (0.05 to 0.20 cc.) for a long period. One of the rabbits, treated with 42.25 cc. of chloroform during a period of one and one-fourth years, did not

give any evidence of pathologic changes in the liver. Jaffé thinks that this one animal got accustomed to chloroform.

Wells [11] supposes that a previous chloroform poisoning acts as a predisposing feature in the later use of chloroform. Davis and Whipple [6] were not able to confirm this statement.

METHODS

Our experiences go still further. We managed to make rabbits bear 1.25 cc. of chloroform injected daily or every other day by means of a careful increase of the doses. We found 1.25 cc. of chloroform to have usually the lethal effect in acute poisoning. We succeeded in giving these large doses to three rabbits by prolonged treatment.

We injected subcutaneously 0.5 cc. of chloroform in two rabbits (Nos. 2 and 3) at the beginning of our experiments. The same rabbits received 0.5 cc. a week after the first injection and the doses given every other day were gradually increased until we reached 1.0 to 1.25 cc. of chloroform. The third rabbit (No. 10) received 1.25 cc. of chloroform in the first injection. After one week's interval it was again injected with increasing amounts from 0.5 to 1.25 cc. during six days; then with another week's interval, the same injections were administered as before. This system of dosage was repeated every other week, totaling forty-one days. At the end of this period we killed the animal.

Rabbit No. 3 received within thirty days 11.8 cc. chloroform. Rabbit No. 2 received within thirty-nine days 22.2 cc. chloroform. Rabbit No. 10 received within forty-one days 11.9 cc. chloroform.

RESULTS OF CHRONIC POISONING

At necropsy we find in rabbits Nos. 2 and 3 a smaller and firmer liver with faded markings and with a smooth and grayish red surface and sections. In rabbit No. 10 we find the liver to be a little firm, normal in size and with a smooth surface; on section the regular liver markings are distinct.

Under the microscope the liver of rabbit No. 3 shows the most pronounced changes. The structure of the liver in comparison with the normal is entirely changed. Necrotic areas are not to be found but only a few scattered hyaline necrotic cells. The periportal

connective tissue is not so prominent as in the liver of normal or of acute chloroform poisoned rabbits. The central veins are in places very wide, while in other places they are hardly visible. In the central half or two-thirds of the lobule, we find the liver cells smaller and more deeply stained than those in the periphery. The nucleus of these cells is not regular, the liver cell columns being formed in places by four to five rows of cells; in other places only by one row. Besides this, there is a connection made by liver cells between the neighboring columns, so that there is a certain net-like arrangement in the central parts of the lobules. The peripheral liver cells largely keep their arrangement, size, shape and staining and in places we find mitotic figures in them. Giant cells are present in a very large number scattered in the central as well as in the peripheral parts. They are located mostly between the liver cell columns and expand them; they are oval in shape and often three or four times as large as a regular liver cell and contain twenty to thirty nuclei. Exceptionally, we find in the giant cells remains of necrotic liver cells. The nuclei in the giant cells are mostly oval, more deeply stained than those of the liver cells and are like those of the endothelial cells. Since the giant cells are located between the liver cell columns, their nuclei are similar to those of the endothelial cells and they have a phagocytic power, it is more than likely that they are cells of endothelial origin. We find, however, such giant cells in close connection with the liver cell columns, and the nuclei are rather similar to those of the liver cells. Therefore, we cannot overlook the observation that these cells may arise from the liver cells. Scattered in the lobule are strikingly large, vacuolated cells. These cells are swollen liver cells with pyknotic nuclei and about two or three times larger than the normal cells. These cells have hardly any cytoplasm which consists of confluent vacuoles. Between the vacuoles the cytoplasm appears like a network of thin fragmentary threads.

The sinusoids lying between the liver cell columns, especially in the central part, are of different widths, so that we find them very narrow and also very dilated, the latter filled with red blood corpuscles. The connective tissue is remarkably increased around the central veins and also in places in other parts of the lobule. Often the connective tissue spreads between the liver cells and separates them from the columns, so that some liver cells are found isolated

in the connective tissue. The periportal connective tissue is also increased, infiltrated with lymphocytes and plasma cells and invades in many places the peripheral part of the lobule. The connective tissue described above does not stain red with van Gieson's stain and has a blue color with Mallory's stain. In these preparations, and also in those stained with phosphotungstic acid hematoxylin, it is easily determinable that the increased connective tissue around the central veins as well as the periportal tissue spreads between the liver cells. With Weigert's stain for elastic fibers, these fibers are visible in the walls of the larger vessels. Some faintly stained elastic fibers are also present in the connective tissue surrounding the central veins. There are more fibers seen by the impregnation method in the periportal connective tissue than normally, and such fibers are in the connective tissue inside the lobule. With impregnation there are black granules present in the paler peripheral cells.

Glycogen is not apparent with Best's carmine stain either in the central and peripheral liver cells or in the large vacuolated cells. Fat stained with scarlet red is visible only in a few liver cells and equally in the central and peripheral part of the lobule. The fat forms minute granules in the cytoplasm, but the nucleus is well stained. We could not demonstrate the fat granules with Nile blue sulphate stain. Fischler's method as well as v. Kossa's silver nitrate reaction gives a negative result. In the liver cells there are in places fine brownish pigment granules which are probably bile pigment. Our efforts to point out iron were without result.

The changes in the liver of rabbit No. 2 are not so well pronounced, so that it is rather similar to a normal rabbit's liver. Necrobiotic cells and mitotic figures are not present at all. Giant cells are fewer in number and large vacuolated cells are more numerous than in the previous case. The increase of the connective tissue in the periportal spaces as well as in the central parts of the lobule is more moderate. Elastic fibers are not visible in the central connective tissue. The scarlet red stain gives the same result as in rabbit No. 3, with the difference that the fat granules are colored violet with Nile blue sulphate. Not even traces of glycogen, lime salt and iron are visible.

The connective tissue increase in the liver of rabbit No. 10 is entirely unremarkable, an increase not being even around the central veins. In the central part of the lobule there are small and deeply

stained liver cells and also a considerable number of large, lightly stained, vacuolated liver cells with pyknotic nuclei. Giant cells are in very small number and mitotic figures are not present at all.

Best's carmine stain gives a striking result. There is a considerable amount of glycogen in the liver cells, even in the small central ones, except in the large vacuolated cells. We find in the central liver cells fine fat granules stained with scarlet red, which take the color of red or violet with the Nile blue sulphate stain. No fat is present in the large vacuolated cells. Fischler's and v. Kossa's methods as well as the iron reaction give a negative result.

DISCUSSION OF CHRONIC POISONING AND CONCLUSIONS

By chronic administration of chloroform, we get entirely different changes from those of the acute poisoning described above. The necrotic processes are not prominent and the necrotic cells are very rare, are partly lying free and partly engulfed in giant cells. But as a proof of previous necrosis, we find a comparatively large number of giant cells which are mostly phagocytic endothelial cells as described above. The appearance of fat droplets in the liver cells is also a sign of the presence of necrobiotic processes which are especially distinct in rabbit No. 10.

The large vacuolated cells in the liver of all these three rabbits, but especially in that of No. 10, have to be dealt with separately. Jaffé [23] points out the presence of these in chloroform poisoning and calls further attention to the absence of fat in these cells. Our experiments confirm his findings and give evidence in addition of the absence of glycogen. This is especially convincing in rabbit No. 10, in the liver cells of which glycogen was found almost everywhere except in the large, vacuolated cells. Jaffé did not find any special localization of these cells in the lobule, although he found them in some cases mostly in the central parts. This is again best shown in rabbit No. 10. Fischer [41] concludes that these cells are the effect of poisons on the liver cells, and it is remarkable that all these poisons are soluble in fat (chloroform, phenylhydrazine, phosphorus, granugenol, etc.). He believes that the increased water content causes the swelling of the cells. As no evidence is given in regard to the increased water supply in the liver affected by these poisons, we have to suppose that the cell membrane is injured by

the poisons soluble in fat, in consequence of which more liquid can reach the inside of the cells. Since the nucleus becomes pyknotic the process has to be regarded as a necrobiotic one. Fischer applies the term "water-poisoning" (*Wasservergiftung*) to this change in the liver cells.

We did not find any glycogen in the liver cells of two rabbits, while the liver of the third rabbit (No. 10) contained large amounts. We cannot give any reason for this as all three animals were kept on the same stock diet. It is remarkable that rabbit No. 10 showed the least marked and the slightest pathologic changes in its liver. This case also supports the connection between the liver changes and the glycogen content, as in acute cases mentioned above.

Out of three chronic poisonings, in one there was hardly any and in the others a slight increase of connective tissue, so that this cannot be classified as an early cirrhotic process. It is evident, therefore, that the prolonged administration of chloroform does not necessarily produce liver cirrhosis.

In rabbit No. 3 we found a transformation of the lobular structure. The increase of the connective tissue arises from two different locations, namely, the areas around the central veins and the perilobular region. Most important and characteristic is the increase of the connective tissue around the central veins, but the perilobular tissue is also increased and is accompanied by infiltration of lymphocytes and plasma cells. The increased connective tissue in both locations spreads in the lobules and seems to fuse together in some places, in consequence of which certain liver cell groups get separated.

These microscopic findings do not exactly cover those of the human atrophic liver cirrhosis. The increase of connective tissue around the central veins is most prominent in chronic chloroform poisoning of rabbits; the changes of the perilobular connective tissue are of secondary importance. Human atrophic cirrhosis shows the contrary of this. The so-called cardiac cirrhosis caused by chronic congestion in humans is the most similar to this experimental cirrhosis. The characteristic features of the cardiac cirrhosis is the destruction of the central liver cells and the increase in the connective tissue around the central veins. According to general belief, the destruction of the cells is due to mechanical means, whereby the sinusoids dilated by chronic congestion compress the central liver cells and

thus destroy them; the destroyed cells are replaced by new formation of connective tissue. Mallory [45] disapproves of this theory. He believes that the destruction of liver cells is not caused by atrophy due to the increased pressure, but produced by the action of various toxins accompanying the chronic congestion. The nutrition of the liver cells is disturbed in chronic congestion and consequently even a very small quantity of weak toxins can produce necrotic changes. The necrotic cells are engulfed by phagocytes and if the regeneration is not complete, the sinusoids are arranged in rays more distinctly around the central veins and form a network filled with blood in the central part of the lobule. This appearance is similar to that of the four and one-half days poisoning described above. The central connective tissue is collapsed and in a later stage is contracted so that the central connective tissue seems to be increased. This microscopic picture is similar in this respect to that observed in rabbit No. 3. The so-called cardiac cirrhosis and the cirrhosis produced by chronic poisoning are similar not only histologically, but also developmentally, because the beginning of both kinds of changes is necrosis of the central liver cells affected by poisons.

Necrosis of the central liver cells, however, does not explain the distinct, if not the excessive infiltration and increase of the perilobular connective tissue. We can suppose that the necrotic liver cells and its decomposed products are eliminated through the lymph vessels and thus they affect the connective tissue around the lobules either as foreign bodies or as poisonous products derived from decomposed liver cells. This statement is supported by the fact that in one case of three days acute poisoning, mitoses were found in the interstitial tissue.

The experimental investigations of chloroform poisoning and its effect on the liver is interesting in several respects. The experimentalist often finds reasons for mysterious human deaths. It is easy to study the necrobiotic processes in the liver. Because of the distinct and widespread changes in the liver of animals poisoned with chloroform, they might be peculiarly adapted to the experimental study of the liver functions. It is especially interesting to draw comparisons between acute yellow atrophy and liver changes caused by chloroform, but we cannot give space for this in this publication. Although no one has succeeded yet in producing changes similar to

human atrophic liver cirrhosis by means of chronic poisoning with chloroform or other liver poisons (alcohol, phosphorus, phenylhydrazine, etc.), these chronic poisonings still are of interesting value in the study of liver regeneration and cirrhosis.

SUMMARY

We studied the necrobiotic processes in the liver by means of subcutaneous injections of 0.5 to 1.25 cc. of chloroform. Three and six hours after the injection, there are no pathologic changes present. Twenty-four hours after the injection and later until four and one-half days well marked necrosis of the central parts of the lobules is visible. The necrosis is hyaline in character with cells having pyknotic nuclei. Very striking is the absence of polymorphonuclear leucocytes. In the livers with extensive necrosis no glycogen is present. A relatively small amount of fat is found in the necrotic cells, but there is a considerable amount of fatty liver cells at the border between the necrotic and normal areas. We find lime salts in the necrotic cells in 80 per cent of the necrotic livers so we consider this characteristic at least in rabbits. In the necrotic areas the endothelial cells are increased in number and form giant cells. The elimination of the necrotic cells is accomplished mostly by the phagocytic action of cells of endothelial origin. Four and one-half days after the poisoning most necrotic cells have disappeared. The regeneration takes place very soon, as a rule on the third day; mitotic figures are regularly seen in the normal liver cells.

In three rabbits the chloroform was administered for longer periods. In these cases no extensive necrosis is present. In the liver of these rabbits we find giant cells and large vacuolated cells in which neither fat nor glycogen is demonstrable. In one of these cases a distinct increase of connective tissue is seen around the central veins and less pronounced in the periportal spaces.

I am indebted to Dr. Fred W. Stewart for his kind assistance in preparing the photomicrographs.

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DESCRIPTION OF PLATES

PLATES 29–32

FIG. 1. Acute poisoning (three and one-half days). Deeply stained necrotic liver cells in the center of the lobule surrounded by a circle of fatty cells. X 100.

FIG. 2. Acute poisoning (four and one-half days). The necrotic cells of the central area are mostly eliminated; the connective tissue and the sinusoids are well seen. No distinct circle of fatty cells. X 100.

FIG. 3. Acute poisoning (three and one-half days). Fatty cells on the border between the necrotic and normal areas. X 450.

FIG. 4. Acute poisoning (three days). Endothelial cells increased in number around necrotic liver cells. X 1000.

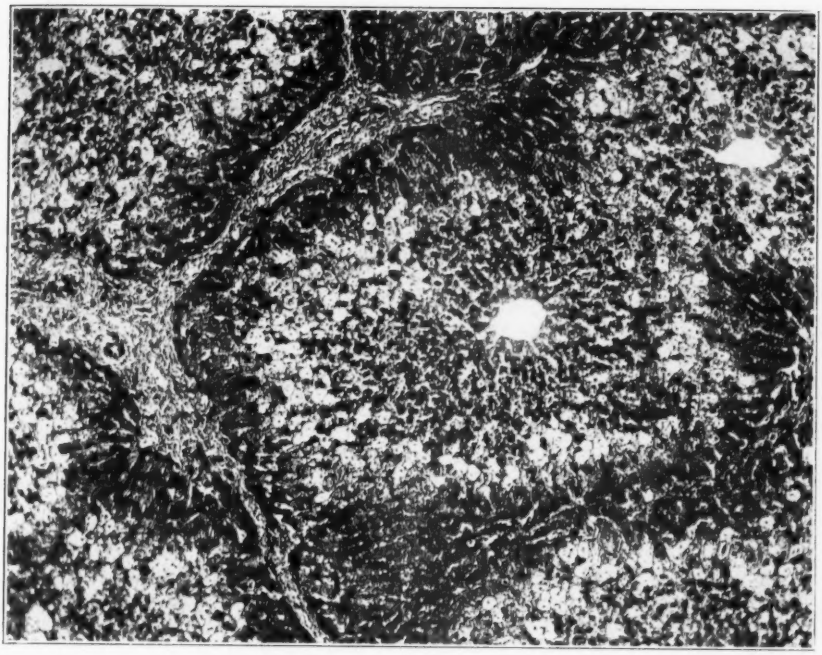
FIG. 5. Chronic poisoning (rabbit No. 3). Giant cell formation. X 1000.

FIG. 6. Acute poisoning (three days). Masses of lime salt colored black with silver nitrate in the necrotic central part of the lobule. X 100.

FIG. 7. Chronic poisoning (rabbit No. 10). No definite necrosis. Large vacuolated cells in the central part of the lobule. X 100.

FIG. 8. Chronic poisoning (rabbit No. 3). No necrosis visible; increased connective tissue between the liver cell columns. X 100.

FIG. 9. Chronic poisoning (rabbit No. 3). Increased perilobular connective tissue spreading in the peripheral part of the lobule. Increased connective tissue around the central vein. X 100.



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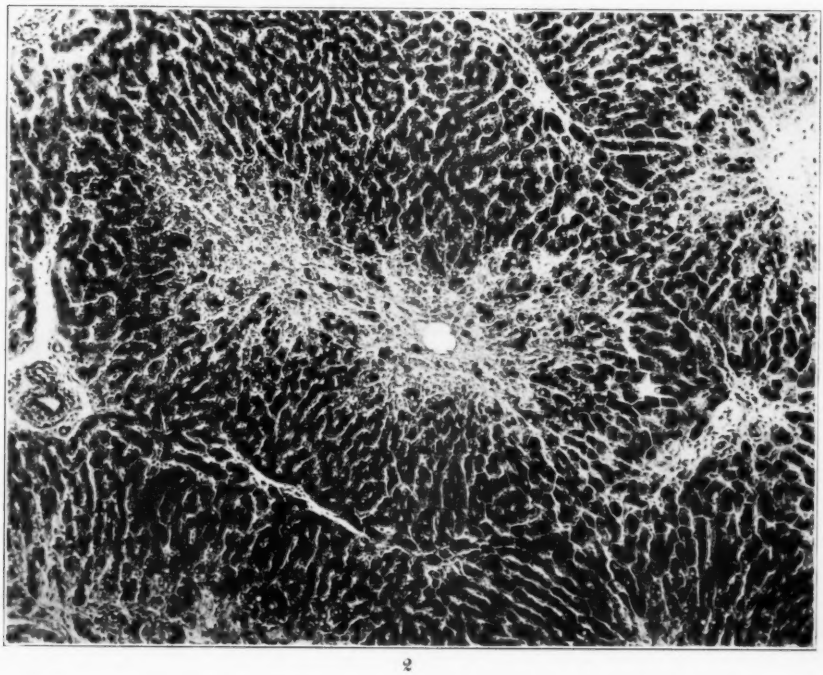

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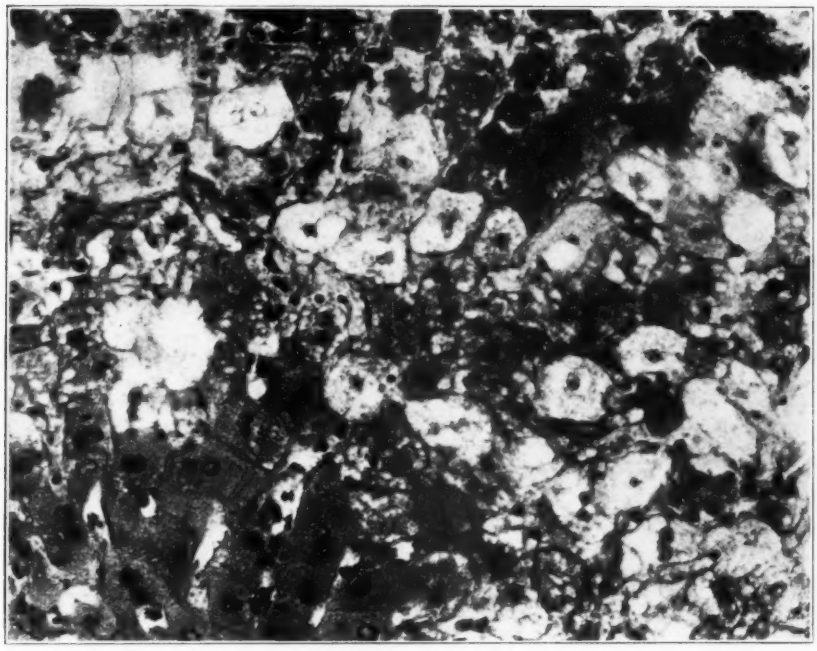
de Zalka

Effect of Chloroform on Liver of Rabbits



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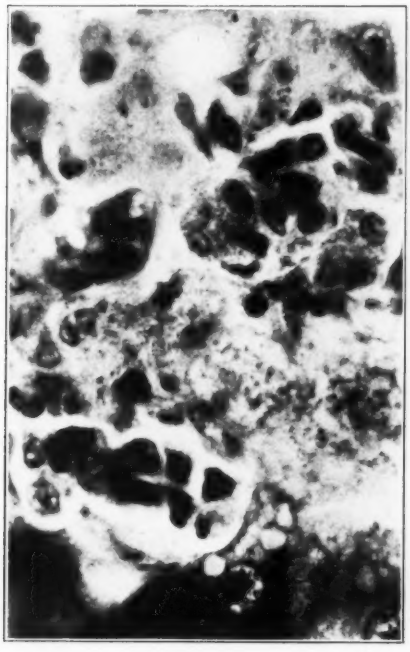

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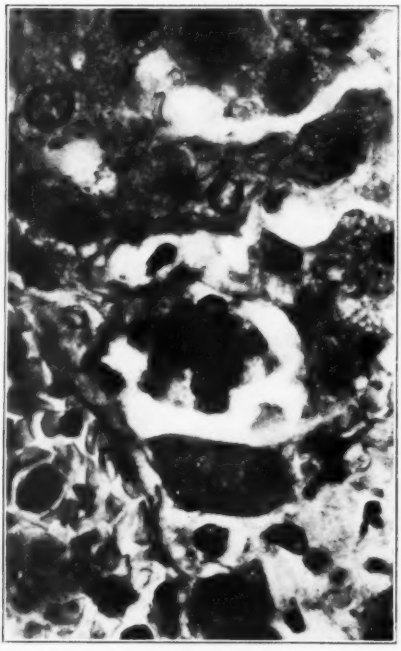

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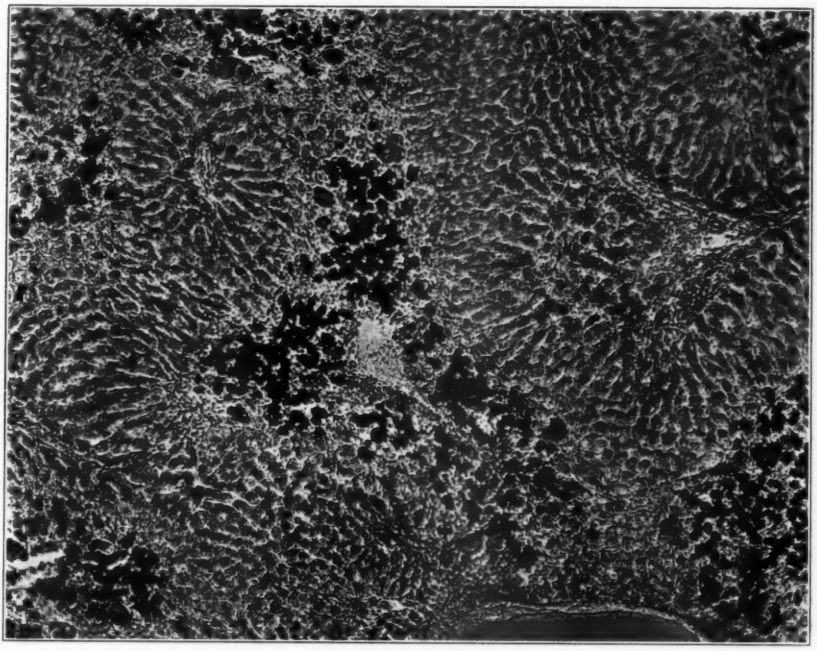
Effect of Chloroform on Liver of Rabbits



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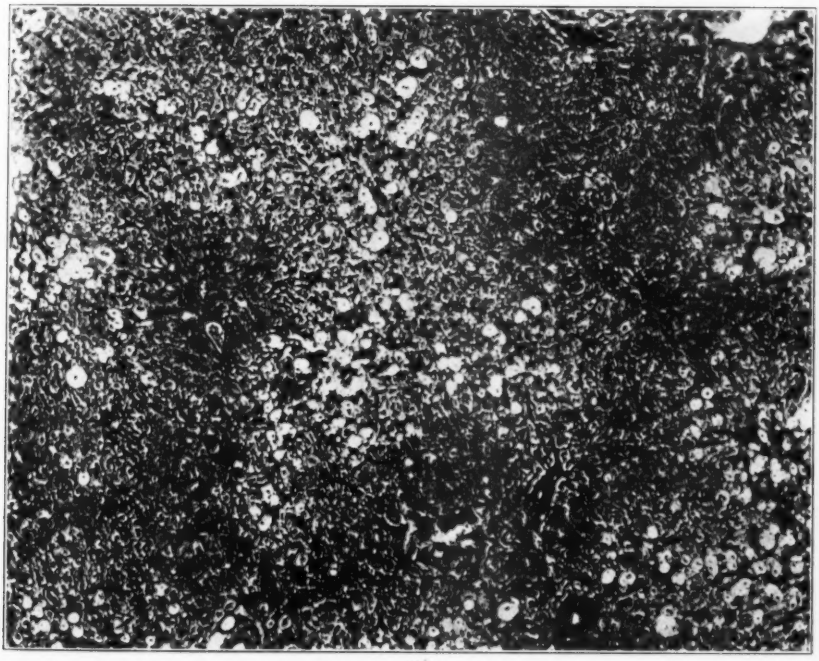

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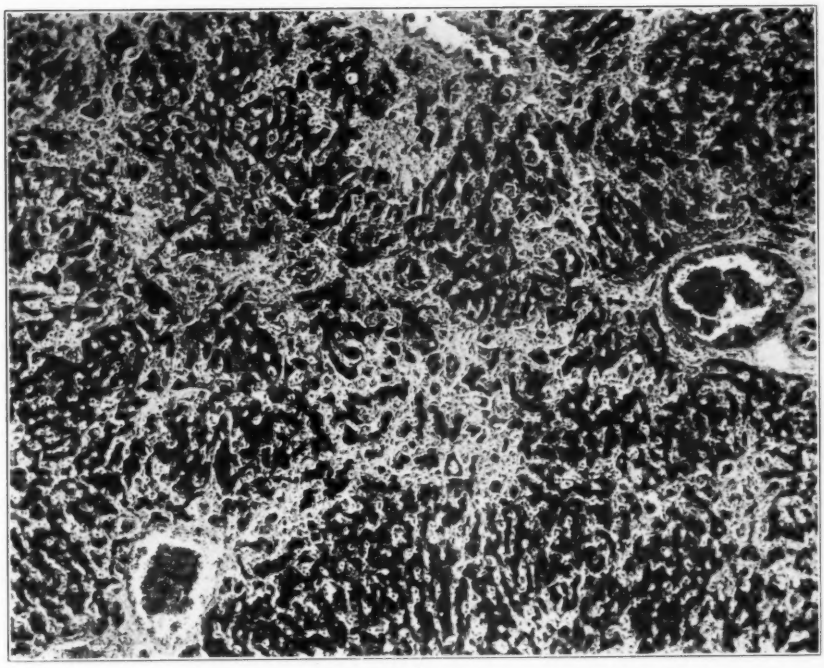
de Zalka Effect of Chloroform on Liver of Rabbits



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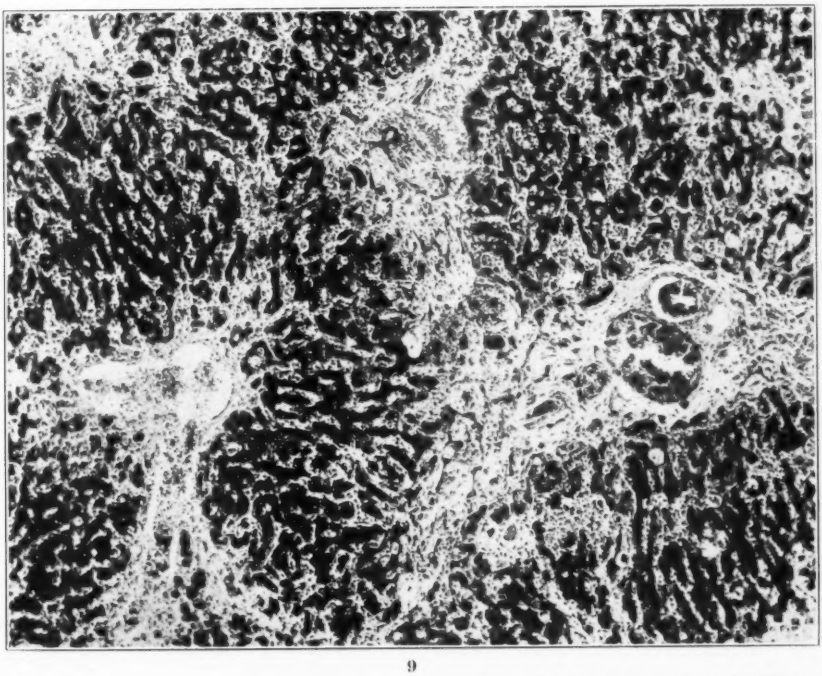

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de Zalka Effect of Chloroform on Liver of Rabbits

FOREIGN PARTICLES AS A CAUSE OF BLOOD CRISES AND ANEMIA *

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While working with suspensions of quartz particles during an investigation on the coagulation of blood, undertaken at the suggestion of Professor John Tait, I made certain incidental observations which are here reported in brief.

When fine particles of quartz are injected intravenously, they are taken up by cells of the "reticulo-endothelial system," as named by Aschoff and Landau [1] (Tait and Cashin [2]). Since erythrocytes [3] and monocytes [4] have in the chick embryo been shown by Sabin to be derived from endothelium, and red blood cells to arise from endothelial cells of adult bone marrow (Sabin [5]), and since particles of quartz are taken up readily by such endothelial cells, it became of interest to know whether these cells after having taken up quartz particles manifest any disturbance of function which can be demonstrated in the peripheral circulation. Accordingly a study was made of the blood cells of a rabbit at various intervals of time before and after injection. It was found that marked changes in the blood picture develop.

About five hours after a single injection of quartz particles into a rabbit, nucleated red cells appear in the circulation, and continue to increase in number for about twenty-four hours by which time they may have reached the number of 4000 per c.mm. If no further injections are made, the nucleated forms gradually disappear from the circulation, and after one or two weeks one sees a nucleated cell only occasionally. This phenomenon seems to correspond to what is known clinically as a "blood crisis." Table 1 illustrates the production of a blood crisis by a single injection of quartz particles.

When the first dose is followed by another, let us say in twenty-four hours, a second blood crisis is superimposed upon the first, the

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increase in number of circulating nucleated red cells depending largely upon the two amounts injected and the interval between the injections. If a third dose is given during the next twenty-four hours, the nucleated red cells still continue to appear in the circulation, though not necessarily in increased number. Of more signifi-

TABLE 1

Weight of rabbit, 2300 gm. Injection of 0.13 gm. per kilo body weight of quartz particles suspended in 6 per cent dextrose solution

Time	Number of red blood cells *per c. mm.*	Number of nucleated red cells *per c. mm.*
Before injection	6,800,000	0
After injection:		
3½ hours	5,990,000	25
7 hours	5,570,000	81
11 hours	6,650,000	165
24 hours	7,700,000	1363
29 hours	6,300,000	4646
48 hours	5,770,000	5640
72 hours	5,780,000	555
96 hours	5,390,000	560
120 hours	5,800,000	996

TABLE 2

Weight of rabbit, 2485 gm.

Time	Number of red blood cells *per c. mm.*	Number of nucleated red blood cells *per c. mm.*
Before injection	6,990,000	0
Dose of 0.1 gm. per kilo body weight administered.		
Time following last injection:		
3 minutes	7,000,000	0
1 hour	6,350,000	0
20 hours	6,210,000	2,400
Same dose repeated at end of 24 hours.		
4½ hours	5,670,000	2,400
23 hours	5,290,000	4,392
Same dose repeated at end of 24 hours.		
24 hours	3,670,000	4,480
28 hours	4,120,000	5,448
50 hours	3,470,000	4,223
82 hours	2,670,000	....
98 hours	1,420,000 1,270,000	....

After the third dose macroblasts and megaloblasts appeared in the circulation. The animal died shortly after the last estimate was made.

cance is the fact that cells of an earlier type of blood formation and of greater relative immaturity appear in the circulation, *i.e.*, macroblasts and megaloblasts. Moreover, a very marked and gradual decrease in the number of circulating red cells commences, in other words a true anemia, which may continue until the animal succumbs.

Table 2 represents a typical case of anemia due to successive intravenous injections of quartz particles.

It has been possible to show that this anemia is due, not to silicon poisoning, but to the presence of the particles. If it resulted from the former one would expect to obtain signs of anemia by using smaller quantities of a more ionizable silicon compound, but such a compound may be given without causing a blood crisis. Moreover, the condition has been produced by various other particles, *e.g.*, India ink. The particles are of course taken up by reticulo-endothelial cells, which we know to be concerned both with blood destruction and with blood formation. The mere presence of the particles in the blood stream is not sufficient to cause a blood crisis and anemia; to produce the effects the particles must reach cells of the so-called reticulo-endothelial system. In the circumstances of the case it must not be supposed that each animal reacts in precisely the same way to a given dose.

A marked decrease in the number of platelets (Burke and Tait [6]) and of leucocytes occurs immediately after an injection, followed in the case of the white cells by a leucocytosis reaching a maximum point in about twenty-four hours.

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